EVALUATION OF IMMUNOGLOBULIN G COMPLEXED FORM OF THYROID STIMULATING HORMONE [MACRO TSH] AS INTERFERENCE IN TSH ASSAY

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APRIL 2018

CERTIFICATE

This is to certify that the dissertation titled, ***"EVALUATION OF IMMUNOGLOBULIN G COMPLEXED FORM OF THYROID STIMULATING HORMONE [MACRO TSH] AS INTERFERENCE IN TSH ASSAY"*** is a genuine work done by **Dr. C.ARCHANA DEVI** for the partial fulfillment of the requirements for M.D (Biochemistry) Branch XIII Examination of The Tamil Nadu Dr. M.G.R. Medical University to be held in April 2018, during the academic period 2015-2018.

Prof.Dr.S.PonnambalaNamasivayam
MD.,D.A.,D.N.B.,
Dean
Stanley Medical College &Hospital,
Chennai – 1.
College&Hospital,

Professor & HOD
Department of Biochemistry
Stanley Medical

Chennai-1

CERTIFICATE BY GUIDE

This is to certify that the dissertation on ***"EVALUATION OF IMMUNOGLOBULIN G COMPLEXED FORM OF THYROID STIMULATING HORMONE [MACRO TSH] AS INTERFERENCE IN TSH ASSAY "*** is a record of research work done by **Dr.C.ARCHANA DEVI** in partial fulfilment for M.D (BIOCHEMISTRY) Examination of The Tamil Nadu Dr. M.G.R. Medical University to be held in April 2018. The period of study is from January 2017 to June 2017.

Prof. Dr.R.SHANTHI M.D., D.C.P.,
Department of Biochemistry,
Government Stanley Medical College,
Chennai - 600 001.

DECLARATION

I, **Dr.C.ARCHANA DEVI**, solemnly declare that the dissertation titled ***"EVALUATION OF IMMUNOGLOBULIN G COMPLEXED FORM OF THYROID STIMULATING HORMONE [MACRO TSH] AS INTERFERENCE IN TSH ASSAY"*** is a bonafide work done by me during the period of JANUARY 2017 to JUNE 2017 at Government Stanley Medical College and Hospital, Chennai under the expert guidance of **Prof. Dr.R.SHANTHI M.D., D.C.P.**, Department Of Biochemistry, Government Stanley Medical College and Hospital, Chennai.

This thesis is submitted to The Tamil Nadu Dr. M.G.R. Medical University in partial fulfillment of the rules and regulations for the M.D. degree examinations in Biochemistry to be held in April 2018.

Chennai-1

Date: **Dr. C.ARCHANA DEVI**

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INDEX

ABBREVIATIONS

TSH – Thyroid stimulating thyroxine

Tg – thyroglobulin (Tg)

PT TSH – pars tuberalis Thyroid stimulating hormone

PD TSH – pars distalis Thyroid stimulating hormone

LD – Long-day (LD) stimulus

TRβ2 – thyroid hormone receptor

MBH – mediobasal hypothalamus

TRH – thyroid releasing hormone

TRHR – thyroid releasing hormone receptor

MTI - melatonin receptors

HAMA – Human anti- mouse monoclonal antibody

RA – Rheumatoid factor

HAb – Heterophile antibodies

IMA – immunometric assays

NABs – naturally occuring antibodies

PEG – poly ethylene glycol

TPA – tripropylamine

TPA – tripropylamine

ECL – electrochemiluminence

INTRODUCTION

INTRODUCTION

Thyroid disorder is one of the most common disease among all endocrine disorders. Measurement of Serum Thyroid Stimulating Hormone (TSH) concentrations are made in most of the laboratories, mainly to screen for primary hypothyroidism. Prevalence of hypothyroidism in the general population ranges from 3.8% - 4.6%. [1] Another epidemiology study in the Indian population suggested the overall prevalence of hypothyroidism to be 10.95%. [2] A follow up survey to determine the incidence and natural history showed an annual incidence of hypothyroidism of 4.1 per 1000 survivors per year in women and 0.6 per 1000 survivors per year in men. [3]. Studies have suggested that subclinical hypothyroidism has a prevalence of 4 to 10% in the general population and the prevalence is found to increase with age and also females more than 45yrs of age have more preponderance. [50]. It has been estimated that about 42 million people in India suffer from thyroid diseases. [4]

Measurement of Serum Thyroid Stimulating Hormone (TSH) levels in the blood sample is the initial best way for the testing of thyroid function. Thus sensitivity and specificity of the thyroid function test is very important. Especially for detecting and treating thyroid diseases, in patients with nonspecific and vague clinical symptoms, the diagnosis and management mainly depends on TSH levels. TSH being an essential laboratory parameter,

its reliability is very important. There are also a number of incidences where TSH values may not correlate with the patient ‘s clinical features. Not all high TSH values are due to true elevation of thyroid stimulating hormone, there are number of causes of interference in thyroid hormone assay. The potential interferences are cross reactivity, drugs, antibodies and macrocomplexes.

Cross reactivity occurs when some endogenous molecules have a similar structure to the measuring analyte, for example TSH and HCG carries an analogue alpha chain that can cause interference. With development of specific monoclonal antibodies cross reactivity has been almost eliminated from modern assays. Other causes of interference include drugs, antibodies and macrocomplexes .[5] . Antibodies which may cause interference in thyroid assay includes HAMA [human anti mouse antibodies], heterophile antibody and rheumatoid factors. However these interferences can be minimised by treating the sample with blocking agents and performing the assay. The occurrence of macrocomplexes is well known with hormones and enzymes like prolactin (macroprolactin), creatine kinase (macrocreatine kinase) and amylase (macro amylase). Recently, cases have been reported with unexplained high TSH values, but clinically euthyroid and they were found to have macro TSH. Prevalence of macro TSH varies from 0.6 to 0.79% [5]. The prevalence of macro-TSH in patients with latent hypothyroidism is 1.62% [6].

Macro hormones are high molecular weight hormones which are conjugated with immunoglobulin, most often occurs as a complex of TSH –

immunoglobulin G. The molecular weight of TSH is approximately 30 kDa and it is easily filtered by kidney, but on combining with an immunoglobulin (IgG) molecule, the molecular weight of this large complex increases to approximately to 200 kDa, which bypasses its filtration by the kidney and leads to accumulation of macroTSH in the serum. [8]. This can cause misleading high values. These macro hormones are biologically inert and lack hormonal activity so, it is assumed that practically no treatment is needed [7]. But the patients may be subjected to thyroid replacement therapy due to spurious elevation of serum TSH values and can even end up in thyrotoxic state.

Macro TSH leads to artefactual TSH elevation which does not correlate clinically and can cause unnecessary repetition of clinical investigation and management [5].

REVIEW OF LITERATURE

REVIEW OF LITERATURE

THYROID GLAND – An overview

ANATOMY

The word ‘thyroid’ was named by WHARTON which means oblong shield [12]. The thyroid gland is in the form of butterfly-shape which is situated in front of the neck just above trachea [13]. The thyroid gland has two lobes connected by the isthmus and a fully formed thyroid gland weighs around 15 to 20 g. Thyroid gland is lined by the thyroid follicle which acts as a secretory unit. The height of thyroid follicle depicts functional activity of thyroid gland. Lacunae which is in the center of follicle lined by the apex of follicular cells is composed of colloid. The composition of colloid is thyroglobulin (Tg) and iodinated thyroalbumin. The important reactions like iodination and the initial phase of hormone secretion, colloid resorption, are believed to take place near the surface of the epithelial cells which plays an important role in of thyroid hormone synthesis. The thyroid gland also has parafollicular or C cells which produce the polypeptide hormone calcitonin. These cells are located within the follicular basement lamina or found in the interfollicular spaces. Vascular supply to thyroid gland is provided by Superior and inferior thyroid arteries and veins. [14]

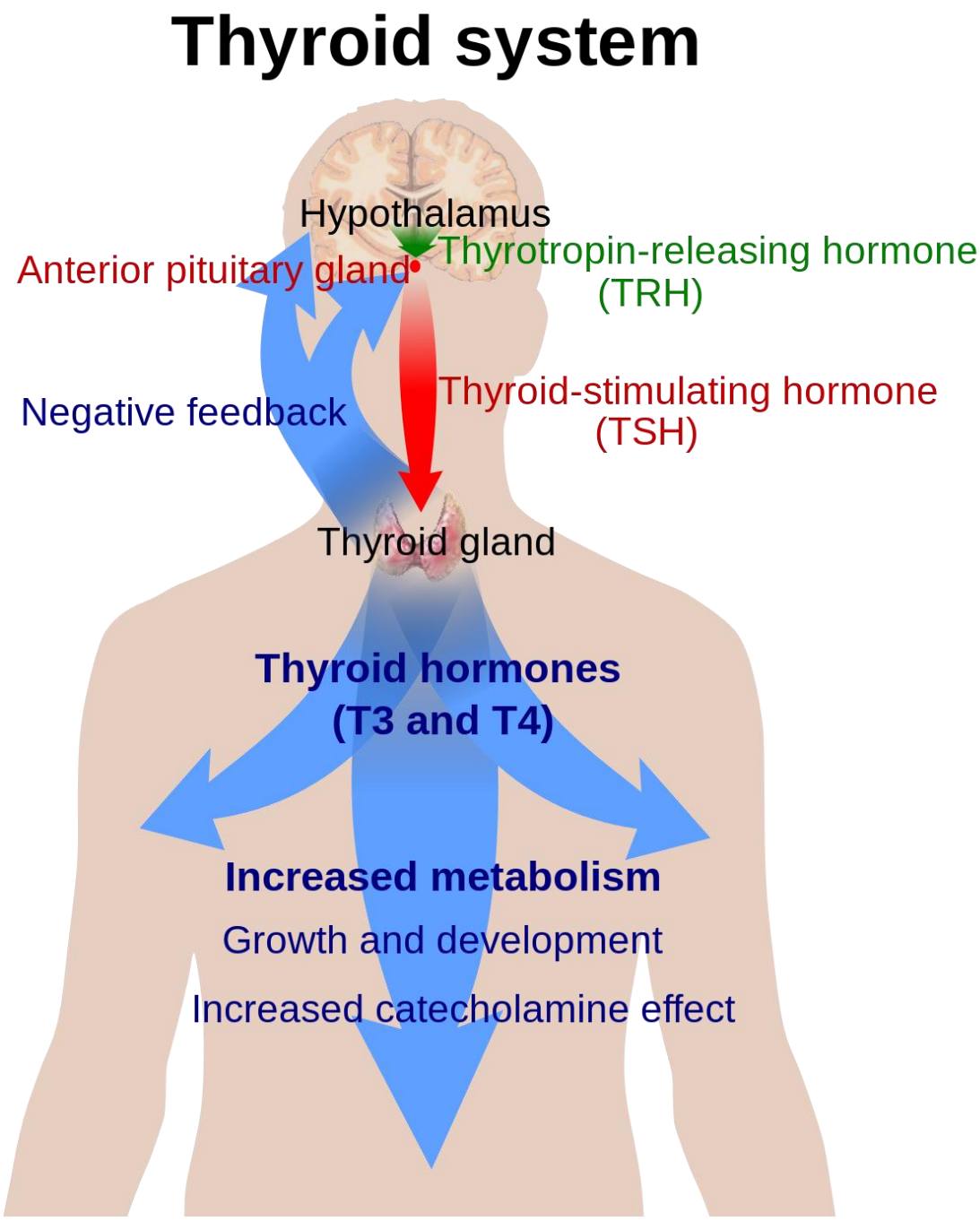

FIG 1 : SHOWING FUNCTIONS OF THYROID HORMONES

FUNCTIONS OF THYROID HORMONE:

Some of the important biological effect of thyroid hormone are :

- it controls basal metabolic rate
- required for normal growth and neural development
- increases adrenergic activity thus increasing heart rate and myocardial contraction
- helps in carbohydrate metabolism and protein synthesis
- increase the synthesis and degradation of cholesterol and triglycerides
- enhances calcium and phosphorus metabolism
- increases the sensitivity of adrenergic receptors to catecholamines

PHYSIOLOGY

Hypothalamic - pituitary -thyroid axis is formed well by 20 weeks of fetal life. The thyrotropin releasing hormone [TRH] from hypothalamus stimulates Thyroid stimulating hormone [TSH, thyrotropin] synthesis.

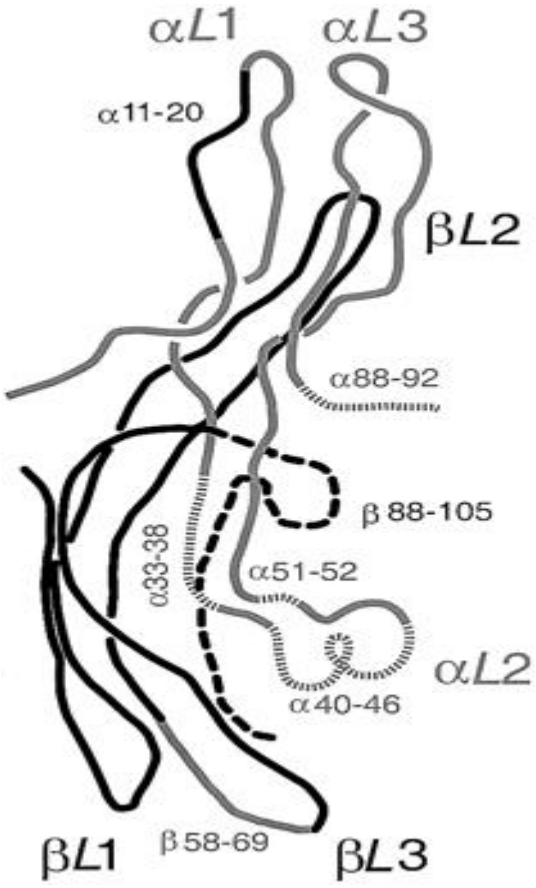

FIG 2: STRUCTURE OF of hTSH . The α-subunit is shown in grey line, and the β-subunit chain is depicted in black line. . The peripheral β-hairpin loops are as: αL1, αL3 in the α-subunit; βL1, βL3 in the β-subunit.

TSH is a glycoprotein secreted from anterior pituitary which in turn stimulates the synthesis of thyroid hormones [15]. TSH is a heterodimeric cystine knot glycoproteins consisting of two noncovalently linked alpha and beta subunits. Alpha subunit is encoded by gene on chromosome 6 whereas beta subunit is encoded by gene on chromosome 1. Alpha subunit of TSH shares a common structure with other hormones like lutenising hormone [LH] and follicle stimulating hormone [FSH], whereas the beta subunit is unique [16]. The pituitary

gland is made up of three lobes: the anterior, intermediate, and posterior lobes. The anterior lobe of pituitary has pars tuberalis [PT], pars distalis [PD] and pars intermedia. The pars distalis of pituitary gland is responsible for synthesis and secretion of TSH -PD which are positively regulated by hypothalamic thyrotropin – releasing hormone [TRH]. Thyroid stimulating hormones secreted from pars distalis of the pituitary [17] act predominantly through thyroid hormone receptor (TRβ2) on thyrocytes to synthesis and release thyroid hormones[TH]. The circulating thyroid hormone formed inturn inhibits PD-TSH by negative feedback mechanism [15].

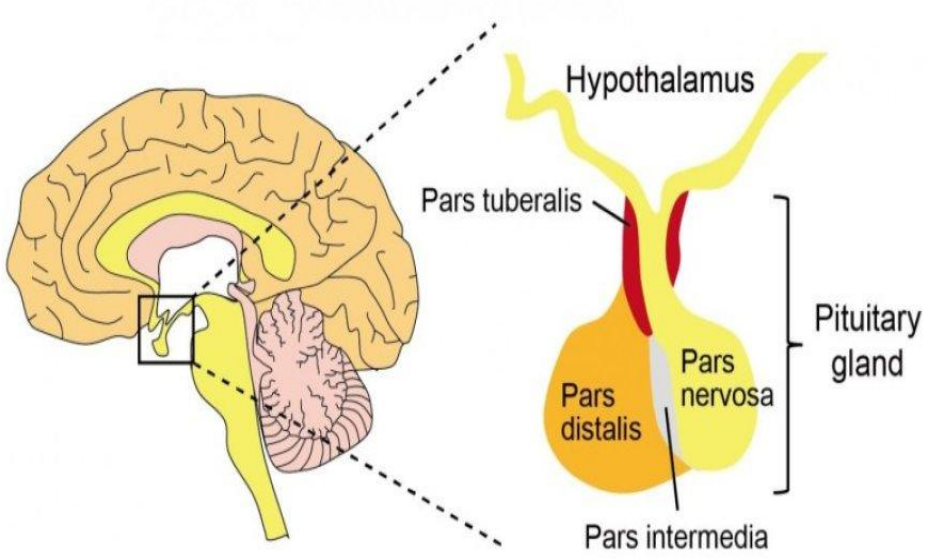

FIG 3 : DIAGRAM SHOWING PARTS OF PITUITARY GLAND.

PT -TSH regulation

PT-TSH is independent of TRH regulation

TSH secreted from pars tuberalis of the pituitary gland plays an important role in regulating seaonality.[18, 19, 20]. The changes in day length regulates some of our physiological activities, like reproduction and migration. Long-day (LD) stimulus induces the production of PT-TSH. This PT- TSH acts on TSHR found in ependymal cells of mediobasal hypothalamus (MBH), which in turn causes expression of *Dio2* gene. *Dio2* gene encodes for type 2 deiodinase. The action of type 2 deiodinase enzyme is that it activates TH and converts the thyroxine (T_4) to bioactive triiodothyronine (T_3) within the MBH, which serves as a key factor for regulation of seasonality. [21]

Tissue specific glycosylation imparts specificity to TSH

Thus in contrast to PD-TSH, the PT-TSH does not take part in regulation of TSH ,the reason behind this is that PT cells lack TRH receptor (TRHR) [22]. But the PT is found to have melatonin receptors [MTI] which is not found in PD [23]. Eyes receive photoperiodic information and transmits it to pineal gland [24]. Pineal gland plays a major role in secretion melatonin in the night which acts as a signal of night length [24]. Thus PT -TSH is influenced by melatonin and photoperiodic informations plays an important part in regulation of TSH in the PT [25].

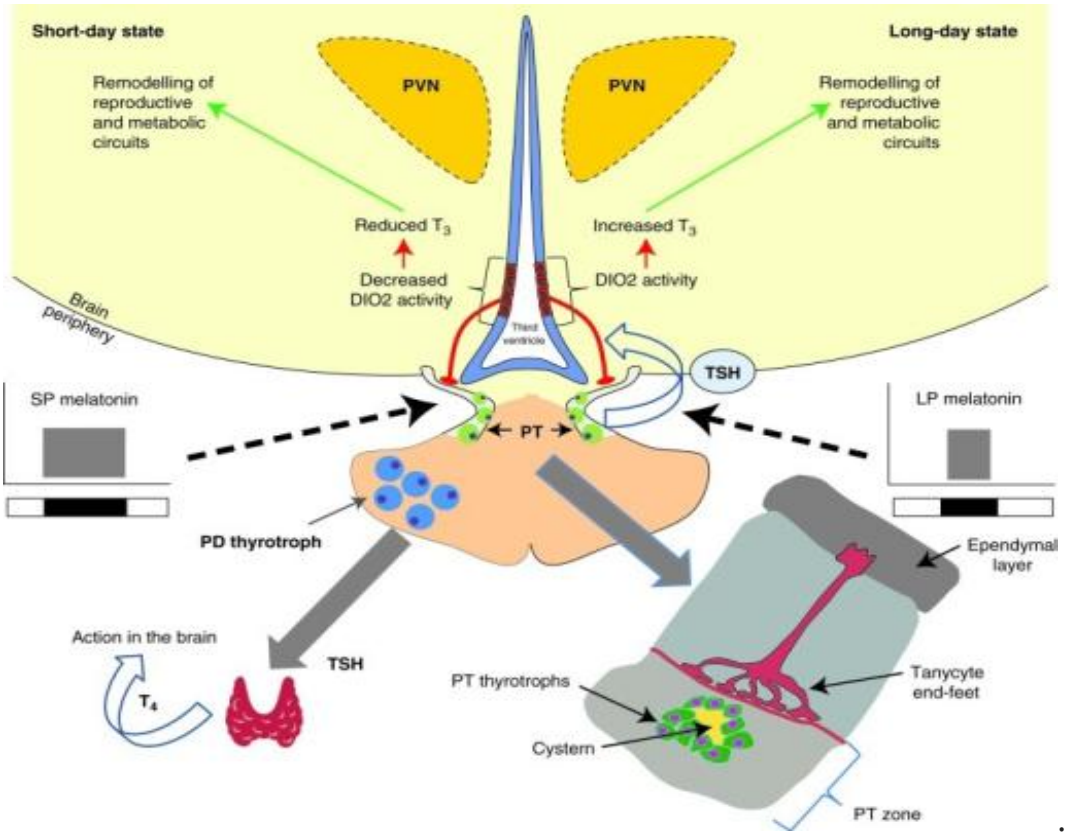

FIG 4: Action of PT TSH. *Photoperiodic information is encoded by the nocturnal melatonin signal generating signals in response to (long) summer day lengths. The prim site of action is the pituitary pars tuberalis. LD activates TSHβ leading to increase in deiodinase 2 activity in adjacent ependymal cells (tanycytes), which has TSH receptor. Thus LD leads to increase of T3, via conversion from T4 to T3 switch now acts on remodelling of reproductive and metabolic processes. TSH-expressing cells*

of the PT does not have TRH receptor, therefore cannot be regulated by a hypothalamic peptide (TRH).

PT-TSH shows less bioactivity :

- PT thyrotrophs distinct from PD thyrotrophs

PT thyrotrophs were distinct from PD thyrotrophs which was revealed by a series of electron microscopic analyses [26, 27] and the TSH concentration inside PD was ~10,000 times greater than that of PT. PD thyrotrophs have large amount of dense secretory granules, when compared with PT thyrotrophs which has only a lesser amount of granules. PT thyrotrophs has a well-formed Golgi apparatus with numerous microvesicles, and lesser amount of secretory granules, that mimics PD thyrotrophs of thyroidectomized rat [28, 29]. The different regulatory mechanisms of PD-TSH and PT-TSH was taken into account for controlling source of TSH in the circulation. Thus, PT thyrotrophs causes release TSH [30, 31] and because serum T_4 concentration has to increase in response to TSH , serum T_4 level was measured. But in contrast serum T_4 levels was not affected by differences in serum TSH, suggesting that PT-TSH has little bioactivity in the circulation.

- **Differential glycosylation of the two TSHs**:The molecular weights of PT-TSH was around ~40 kDa and that of PD-TSH was ~37 kDa [15]. Differential glycosylation of TSH was one the main reason for the difference in their molecular weight. In PD TSH, glycans were mainly biantennary and sulfated complexes [32]. Though PT-TSH shares few of these biantennary *N*-glycans, tetra-antennary and tri-antennary multi-branched *N*-glycans linked together with sialic acid were also found in the PT. Because negatively charged sulfates affect the intensity of the positive-ion mass, spectrograph [32] quantitation of *N*-glycan is relatively difficult. The tissue-specific expression of glycosyltransferases causes the difference in oligosaccharides between PD- and PT-TSH. This tissue specific glycosylation of PT is the cause of the low bioactivity of PT-TSH in the peripheral circulation **[15]. This TSH can bind to serum immunoglobulin (IgG) to form a higher molecular-weight complex known as "macro-TSH" which has little bioactivity in humans and rats [33].**

The molecular weight of TSH is approximately 30 kDa, but when it combines with immunoglobulin (IgG) it forms a high molecular-weight complex of approximately 200 kDa, which bypasses its filtration by the kidney and leads to accumulation of macroTSH in the serum. [8]

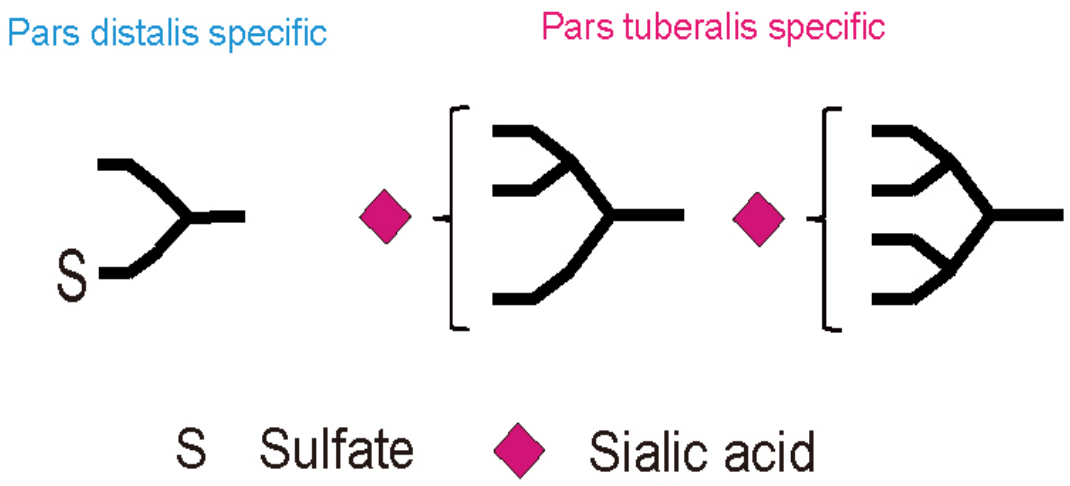

FIG 5: .STRUCTURE OF PD - TSH AND PT- TSH.

PD TSH , glycans were mainly biantennary and sulfated complexes

PT-TSH. tetra-antennary and tri-antennary multi-branched *N*-glycans linked together with sialic acid

HYPOTHYROIDISM

Hypothyroidism is a very common thyroid disorder due to thyroid hormone deficiency, which can be easily diagnosed and managed, but can be potentially fatal when left untreated. It is found in 2% to 15% of the population among which women are more frequently affected than men. The statistical reference ranges of the relevant biochemical parameters plays a crucial role in defining hypothyroidism.

Based on TSH and FT4 values hypothyroidism is classified as

- Primary hypothyroidism
- Central hypothyroidism

PRIMARY HYPOTHYROIDISM

In primary hypothyroidism, TSH is raised with low levels of free thyroxine (FT4) which is mainly due to primary thyroid gland failure. The primary hypothyroid may be caused due to endogenous and exogenous factors. Endogenous causes are autoimmune thyroiditis, inborn errors and developmental anomalies. Exogenous factors causing hypothyroidism are, either iodine deficiency or excess, drugs, goitrogens, post surgical or following radioactive iodine treatment. In primary hypothyroidism the feedback mechanism causes compensatory thyroid enlargement (goiter) through the hypersecretion of thyrotropin-releasing hormone (TRH) and TSH due to decreased thyroid hormones. Primary nongoitrous hypothyroidism is due to loss or atrophy of thyroid tissue, which results in decreased production of thyroid hormones inspite of maximum stimulation by TSH. Hashimoto thyroiditis is the most common type of primary hypothyroidism in developed countries where iodine intake is sufficient, Whereas in under developed and developing countries, the iodine deficiency is the common cause of goitre in hypothyroidism. The commonest cause of nongoitrous hypothyroidism is after thyroidectomy or after radio-ablation of the thyroid gland in case of Graves

disease. Primary hypothyroidism is most commonly associated with circulating antithyroid antibodies which can be found in coexistence with other diseases in which autoantibodies are present. In addition, primary hypothyroidism can be one of the manifestation of an autoimmune syndromes. Thus impaired T3, and T4, leading to increased secretion of pituitary TSH cause remarkable increase in serum TSH concentrations. Hence elevated concentration of TSH is an important laboratory finding in the early detection of thyroid disorders.

CENTRAL HYPOTHYROIDISM

Central hypothyroidism is due to pituitary disorders which can be caused by tumours, malformation or post infection. Central hypothyroidism can be secondary hypothyroidism due to TSH deficiency or tertiary hypothyroidism due to hypothalamic disorder from thyroid releasing hormone deficiency . Central hypothyroidism occurs either due to hypothalamic or pituitary diseases that can produce a deficiency in TRH, TSH, or both. Isolated TSH deficiency is very rare, and most often patients with secondary hypothyroidism have associated pituitary hormone deficiencies as well as panhypopituitarism. In secondary hypothyroidism, the serum concentration of thyroid hormone is low, and TSH concentrations is either low or within normal range. When T4 and TSH concentrations are both low, a TRH test measurement can help in the diagnosis. In patients with destructive lesions of the pituitary gland that result in TSH deficiency, there is no response to TSH with exogenous TRH

administration. In patients with hypothalamic defect the peak TSH response to TRH can be normal, but can have a delay 45 or 60 minutes after the TRH administration when compared to the usual time of 20 to 30 minutes.

SUBCLINICAL HYPOTHYROIDISM

Subclinical hypothyroidism has serum thyroid-stimulating hormone (TSH) level above the upper limit of normal inspite of normal levels of serum free thyroxine [78].

Subclinical hypothyroidism (SCH) is diagnosed when the serum thyroid stimulating hormone (TSH) level is consistenly high for 6 to 12 weeks with FT4 concentration within normal reference range. Causes of subclinical hypothyroidism are recovery from non thyroidal illness, positively interfering antibodies , suboptimal treatment of primary hypothyroid or poor patient compliance with the thyroid replacement. Previous studies suggested that, 51% of 3775 patients with TSH 5.5–10 mIU/L were found to have TSH levels within the reference range when they were remeasured 5 years later [48,49,] without any treatment. Therefore routine treatment of subclinical hypothyroidism is still controversial. But experts have suggested thyroid replacement therapy is essential for patients with subclinical hypothyroidism with TSH 10 mIU/L or greater.

CLINICAL SYMPTOMS

The most common symptoms in adults are tiredness, lethargy, cold intolerance, constipation, horseness of voice, weight gain, and dry skin. Sometimes the symptoms are vague and generalized that they can be missed.

Thus statistical reference ranges of biochemical parameters like TSH plays a crucial role in defining hypothyroidism. The standard treatment is thyroid hormone replacement therapy with levothyroxine.

TSH MEASUREMENT.

Serum TSH as a single hormone , is one of the most sensitive index for detection of thyroid abnormalities [51]. The current standard of care has even more improved with upcoming of automated immunochemiluminometric assays for TSH which are ultrasensitive [54]. The assay has a sensitivity of 0.004 mIU/L.

Measurement of TSH plays a crucial role in newborn screening, work up of female infertility, in monitoring thyroid replacement therapy and in screening adults for other thyroid diseases.

Misleading TSH value :

The diagnosis of TSH values can be misleading due various interfering factors. They can be either due to biological or technical factors .

Biological factors are :

- Unstable thyroid status When the thyroid hormone replacement therapy is suboptimal or in case of poor patient's compliance there is a lag in resetting of pituitary TSH, during such period there is TSH instability which can be misleading.
- TSH secreting tumours like adenomas
- Central hypothyroidism
- Hypothalamic dysfunction
- Resistance to thyroid hormone

Technical factors :

There are various interferences that can contribute to errors in the laboratory values. The effects of interferences like lipemia, hemolysis and bilirubinemia are well known for all assays. Immunoassays are mainly subjected to antigen-antibody interactions that alter and cause interference in the measurement of analyte.

Endogenous antibodies are known to bind or block the binding sites present on the capture and signal antibodies , which can cause falsely high values. Some of the endogenous antibodies are heterophile antibodies and rheumatoid factors. The most common autoantibodies are

- antibodies to thyroglobulin,
- antibodies to microsomal thyroid peroxidase,
- antibodies to the TSH receptor ,
- antibodies to T4 and T3 .
- Antibodies against TSH

Technical factors due to endogenous antibodies are one of the most common causes of a false elevation TSH [52,53].

Antibodies can be:

- Antibodies against assay antibodies eg. Heterophile antibody
- Antibodies against analyte eg: macro TSH
- Antibodies against signal molecules eg: anti-Rhuthenium antibodies.

Heterophile antibodies (HAbs).

Heterophile antibodies are weak antibodies which are generally non specific and directed against poorly defined antigens. Human anti- mouse monoclonal antibody (HAMA) and rheumatoid factor (RF) are two important heterophile antibodies that can cause interference in TSH assay. The prevalence of HAbs is 30-40 percent [55] and have the potential to interfere with methods that use immunometric assays (IMA)[56].

ENDOGENOUS ANTIBODIES CAUSING INTERFERENCE

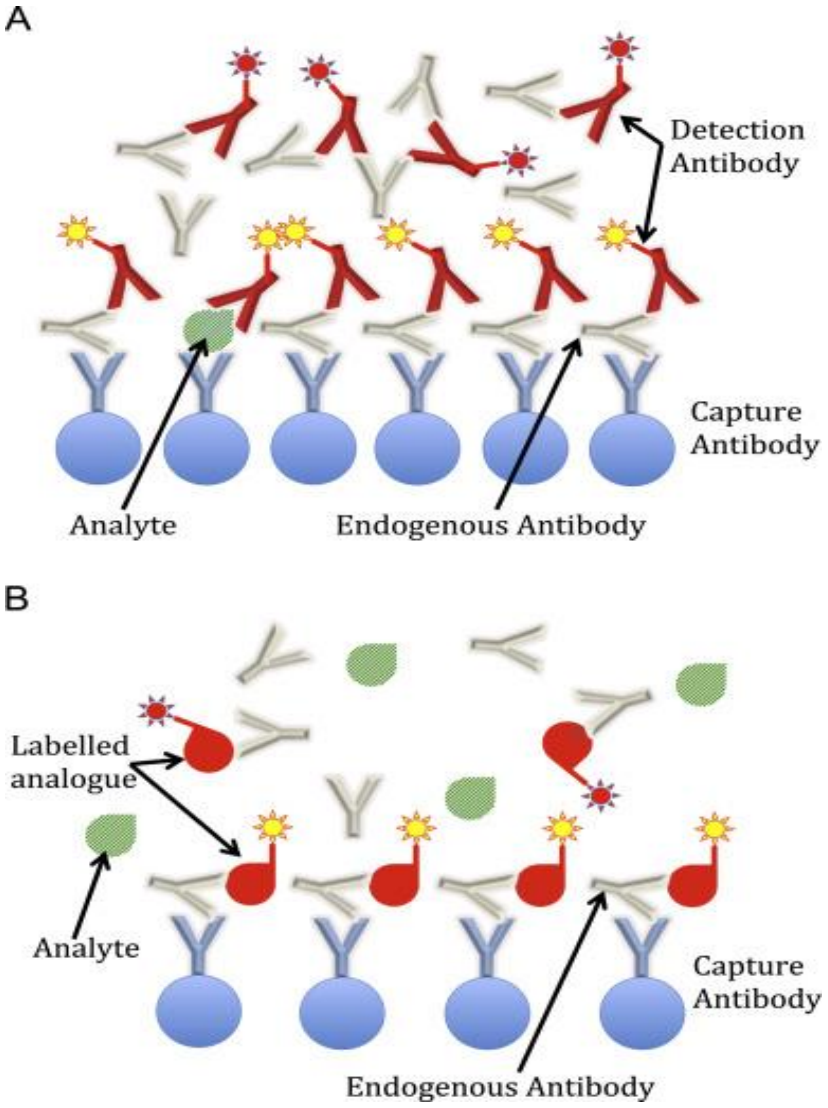

FIG 6 : . Falsely elevated measurements due to interference from heterophile antibodies.

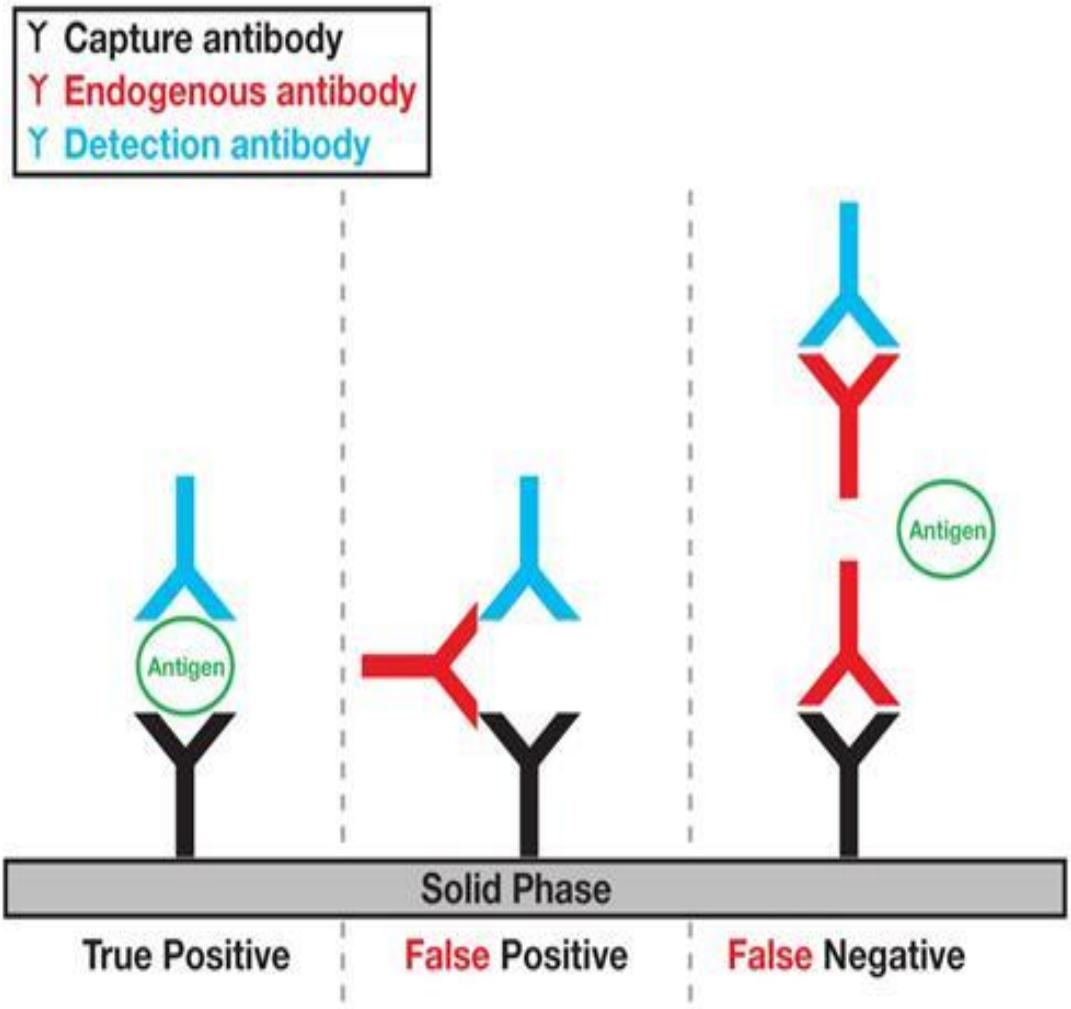

FIG 7: . ENDOGENOUS ANTIBODIES ARE PRODUCED AGAINST POORLY DEFINED ANTIGENS .THEY CAN CAUSE FALSE POSITIVE OR NEGATIVE RESULTS

Heterophile antibodies (HAb), which are human poly-specific antibodies that targets against animal antigens,among which the human anti-mouse antibodies (HAMA) is most common one [57].HAMA is found to occur in 40% - 70% of patients treated with mouse monoclonal antibody and it can occur in 10 % of the general population. HAb can also target human antigens [58] like rheumatoid factor (RF), an immunoglobulin which is commonly associated with autoimmune conditions [59]. RF can interfere with free and total thyroid hormone tests, TSH [60] and Tg . RF factor is found to occur in 70% of the cases with autoimmune rheumatic disease and it can occur in 5 - 10 % of the general population. Rheumatoid factor (RF) is an immunoglobulin which has reactivity to the Fc portion of human IgG. Some RhF species which is also known to cross-react with animal immunoglobulins have the potential to act like heterophile antibodies. It is documented that the patients who have received vaccines recently , blood transfusions or those who have received monoclonal antibodies for treatment, as well as veterinarians and those people who are exposed to close contact with animals, are more prone to the interferences caused by HAb and HAMA [61,62]. When HAMA interference is suspected, the patient's serum can be preincubated for 1 hr at room temperature with non immune mouse serum. After this absorption procedure, the assay can be carried out, to eliminate the interference.

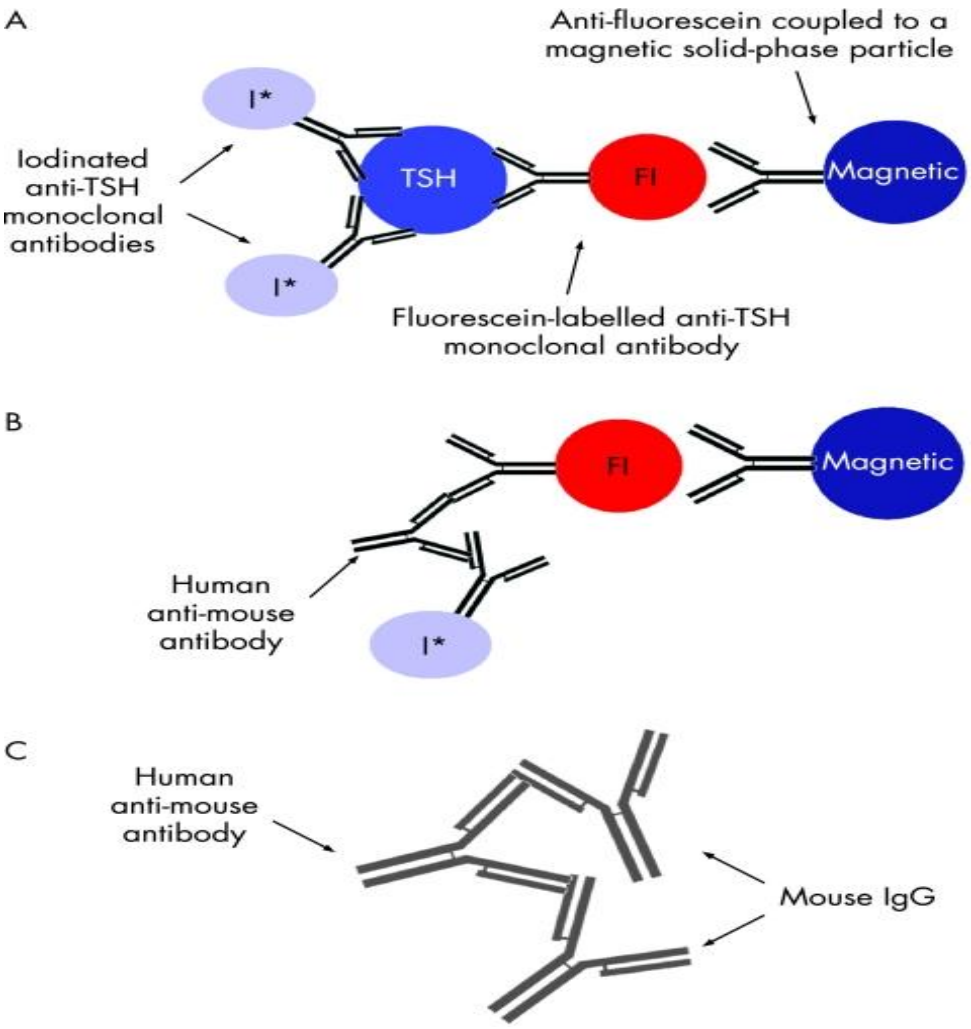

FIG 8: . HAMA interference in TSH assay.

Commercial HAMA blocking reagents are available to counteract heterophile antibody interferences, as well as heterophilic blocking tubes, heterophile- blocking reagents, and non- specific antibody- blocking tubes are also available.

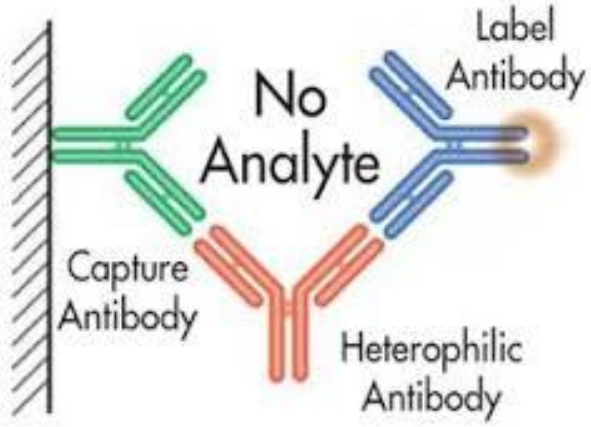

FIG 9: . INTERFERENCE OF HETEROPHILE ANTIBODY, SHOWS FALSE POSITIVE RESULTS IN SANDWICH ASSAY.

Anti-Reagent Antibody *:*

Some patients develop antibodies that are targeted against test reagents like Rhuthenium that cause interference with TSH . Anti rhuthenium antibodies is known to occur in areas of textile industries where rhuthenium was used for dying process of the cloth. It should also be considered that the anti-Rhuthenium antibodies of different patients may differ with each analyte to different degrees [63,64].

Antibodies against Streptavidin-Biotin:

Interferences also occur from antibodies targeting against either Streptavidin [65] or biotin reagents [66]. Reports have suggested that patients with

high dose of biotin ingestion are known to produce interference in TSH assay.[67,68].

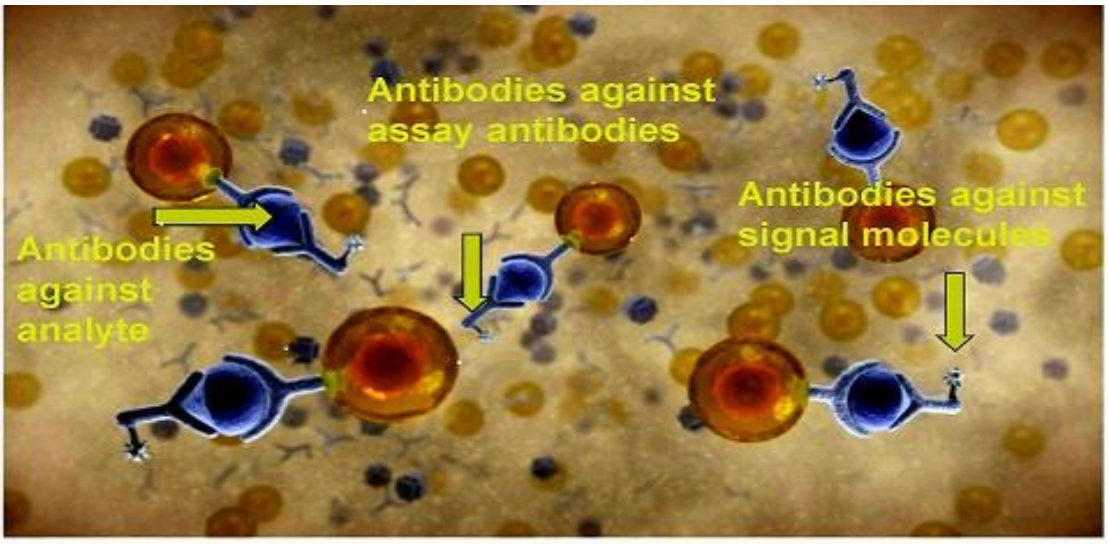

FIG 10 : . ENDOGENOUS ANTIBODIES

TSH Variants.

TSH variants is one of the rare cause of interference [69]. Nine different types of TSH beta variants have been found . [70]. These mutant TSH can exhibit altered immunoactivity that can be detected by few of TSH assay methods but not by others [69]. The bioactivity of the TSH mutants shows a range of bioactivity that varies from normal to bio-inert, this results in discordances between the TSH value and clinical status of the patient [69] and also discordance

between TSH/FT4 values [70]. These TSH genetic variants play a major role in occurence of congenital hypothyroidism [71].

MACRO TSH.

Macro TSH is one of the reason for misleading high TSH values . The prevalence of macro TSH is approximately 0.6 percent, but can be as high as ~1.6 percent in patients with subclinical hypothyroidism.

MACRO TSH – AN OVERVIEW

PT TSH which has tetra-antennary, tri-antennary multi-branched *N*-glycans linked with sialic acid which can conjugate with immunoglobulin, to form a complex of TSH – immunoglobulin G [15]. Macro TSH is found to complex with immunoglobulin G molecules that are found to occur as naturally occurring antibodies [NABs], in contrast to the antibodies induced by exogenous antigen [34]. Ig M is the primary immunoglobulin which produces NABs but Ig G is also known to produce NABs [35].PT TSH formed macro TSH mainly binds with Ig G 2b class of immunoglobulin [15]. TSH is also know to form complexes with albumin [15].

Macro TSH is found to have:

- High molecular weight.
- High stability.

- Long half – life
- Low bioactivity.

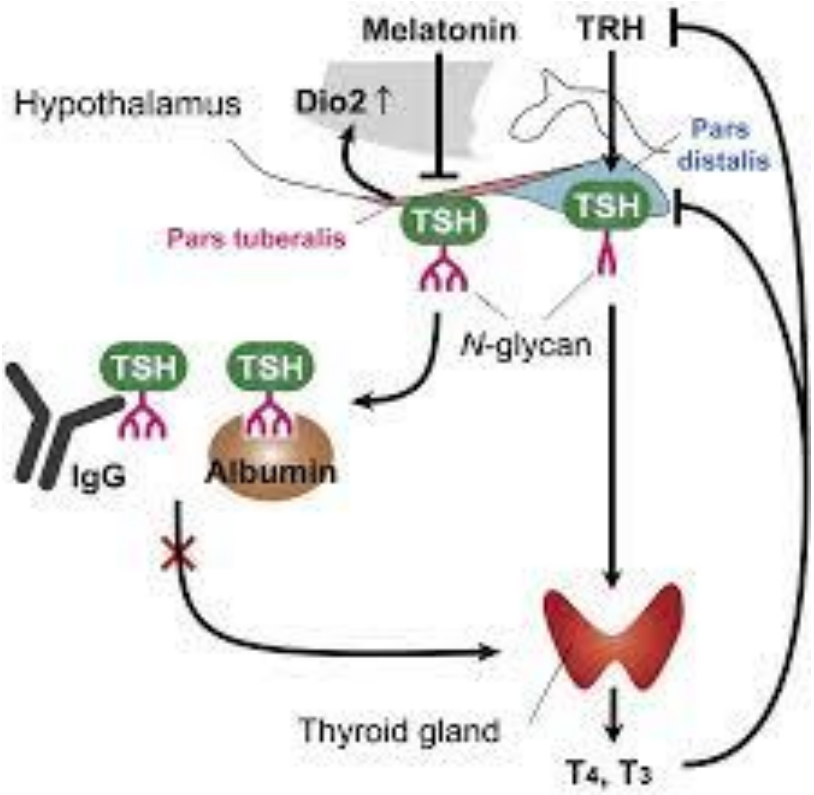

FIG 11: . REGULATION OF TSH , FORMATION OF IgG – TSH COMPLEX TSH is a hormone secreted by the pars distalis (PD-TSH) that stimulates the thyroid gland to synthesize and secrete thyroid hormones, which regulate growth and metabolism in the body. Hormones affect the target organ through circulation in the bloodstream. PD-TSH is regulated by the thyrotropin-releasing hormone (TRH) secreted by the hypothalamus, whereas PT-TSH was not controlled by TRH but is being controlled by a hormone called

melatonin, which is a hormone secreted by the pineal gland during the night.

PT TSH which has a molecular weight of around ~40 kDa can conjugate with immunoglobulin G molecules to form a large complex of approximately 200 kda . This large molecular weight complex is not filtered by kidney which causes accumulation of macro TSH. The complex of TSH Ig - G is biologically inert but the immmunoreactive property is intact so it can be measurable which can give a false elevation of TSH values without the patient being symptomatic[8].

The TSH Ig - G complex is found to be very stable and the PT TSH binds with the IgG for a longer time in the circulation. Acidification of macro TSH below pH 3 has been known to dissociate the macro-TSH complex in humans [36,37]. But in contrast acidification of the stable mouse IgG-TSH complex does not dissociate the IgG-TSH complex.[15] .

Glycosylation plays a major role in contributing to the half-life of circulating glycoprotein [38,39.] TSH with shorter half-life (~0.5–1 h) have a faster hepatic clearance. Hepatic receptors are found to interact with sulfated TSH, which causes pulsatile secretion of the TSH level in serum.[38,39,40]. For example, even though both LH and FSH shows regulation by pulsatile GnRH secretion, it is only LH that shows episodic variation. The reason behind this is

FSH has sialylated *N*-glycan and lacks the sulfate modification, which results in a long half-life of ~17 h [41]. Likewise, sialylated TSH is excluded from specific receptor-mediated clearance from the liver [40]. Therefore PT-TSH, which has multi-antennary sialylated *N*-glycans has a longer half-life than PD-TSH which is one of the main reason for the longer half life of macro TSH. It was found that rodents and patients with hypothalamic hypothyroidism shows increased levels of circulating TSH with altered oligosaccharides [42,43].

Hypothalamic hypothyroidism can cause the appearance of multi-antennary *N*-glycans in circulating TSH [44]. In thyroidectomized or thyroid tumor-induced hypothyroid mice sialylation and multi-antennary glycans were found to be increased, and sulfation to be reduced in circulating TSH [45,46.] The gene encoding for α2–3 sialyltransferase also plays an important role in increasing sialylation of TSH during hypothyroidism [47].

VARIOUS METHODS FOR CONFIRMING INTERFERENCES ARE:

- The assay can be repeated with an alternative immunoassay platform, preferably using assay antibodies from a different species

- Sample treatment with polyethylene glycol.

- Sample treatment with protein G or A .

- Serial dilution of the samples showing non linearity indicates assay interference

PEG PRECIPITATION TEST

PEG is polyethylene glycol which is water soluble nonionic synthetic polymer with an empirical formula $HOCH_2(CH_2OCH_2)mCH_2OH$. This was first introduced by polson et al in 1964 for precipitating proteins[72].PEG precipitation is a very simple and cost effective method for screening antigen antibody interference that can be routinely be used in laboratories . PEG precipitates immunoglobulins G, A and M. This method can be used as a simple screening test for patients suspected to have macro TSH .

PROPERTIES OF POLETHYLENE GLYCOL[PEG].

Physical Property*					
PEG	Average value of m	Average molecular weight	Freezing/ Melting point (°C)	Viscosity at 99°C (cSt)	Nature
400	8.7	380-420	4-8	7.4	Liquid
600	13.2	570-613	15-25	11.0	Liquid
1450	32.5	1300-1600	44-48	25-32	Solid
3350	75.7	3000-3600	48-54	76-110	Solid
8000	181.4	7000-9000	60-63	470-900	Solid

*Compiled from references 1 and 2.

MECHANISH OF ACTION OF PEG

There is a positive perturbations of the chemical potentials of the proteins on adding PEG , there is an interactions between PEG and proteins in aqueous medium which is repulsive in nature and this repulsion becomes stronger as the molecular size of PEG increases [74]. Such an unfavourable interaction can be relieved by the formation of interprotein contacts which can reduce the surface area of protein that are exposed to the solvent and thus can reduce the interactions. This can be achieved by either protein self association or precipitation [73.]

POLYMER EFFECT

Steric exclusion explains effect of polymers on protein solubility.

$$V_T = V' + V_E$$

- V_T - Assuming total volume of solvent occupied by polymer and protein
- V_E – volume occupied by polymer (excluded volume not occupied by protein)
- V' – volume occupied by protein

Increased V_e caused by increase in number or size polymer will decrease V ´and effective increase in concentration of protein molecule. Probability of collision and self association increased and large insoluble aggregates formed.

PEG is used for separation of free hormones from those bound to antibodies thus, helping as a screening tool for detecting macro TSH. Samples with a recovery less than 25% using PEG precipitation method are considered to have antigen antibody interferences .

PROTEIN G ADDITION TEST

Samples with recovery less than 25% are further subjected to protein G addition test. Protein G agarose gel absorbs only IgG complexed form of TSH, so removes most of the TSH which exist in the form of macro TSH which is mostly immunoglobulin G complexed form of TSH

So this study is aimed to evaluate the possibility of macroTSH as an interference in the measurement and interpretation of TSH which may cause a false elevation of TSH values

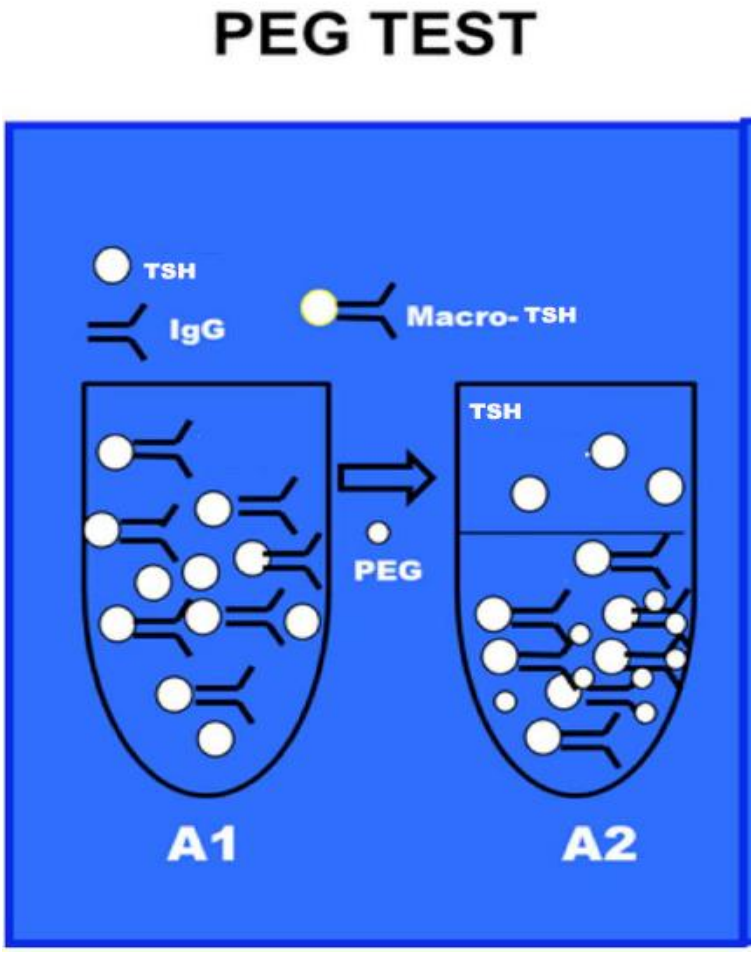

FIG 12: . Adding PEG to the serum of a subject with Macro-TSH (A1) causes the precipitation of Macro-TSH complexes, while unbound enzymes stay in supernatant (A2). The supernatant measured for TSH values in ECLIA.

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AIM AND OBJECTIVES

AIM AND OBJECTIVES

AIM:

To evaluate the interference of immunoglobin G complexed form of thyroid stimulating hormone [macro TSH] in TSH assays.

OBJECTIVES:

- TSH more than 10 µIU /mL and FT4 within normal range [0.8 - 2.7ng/dl], are evaluated for analyte –antibody interference by PEG precipitation method.
- Samples are evaluated to confirm the presence of IgG complexed -TSH by protein G addition test.

MATERIALS AND METHODS

MATERIALS AND METHODS

This study was conducted in government Stanley medical college hospital to evaluate immunoglobulin G complexed form of thyroid stimulating hormone [macro TSH] as interference in TSH assay.

STUDY CENTRE :

Government Stanley medical college hospital , Chennai -1

DURATION OF STUDY :

6 months (January 2017 to June 2017)

STUDY DESIGN:

Cross sectional study

INCLUSION CRITERIA:

Patients with TSH more than 10 mU/L and Free T4 within normal limits [0.8 -2.7ng/dl]

EXCLUSION CRITERIA:

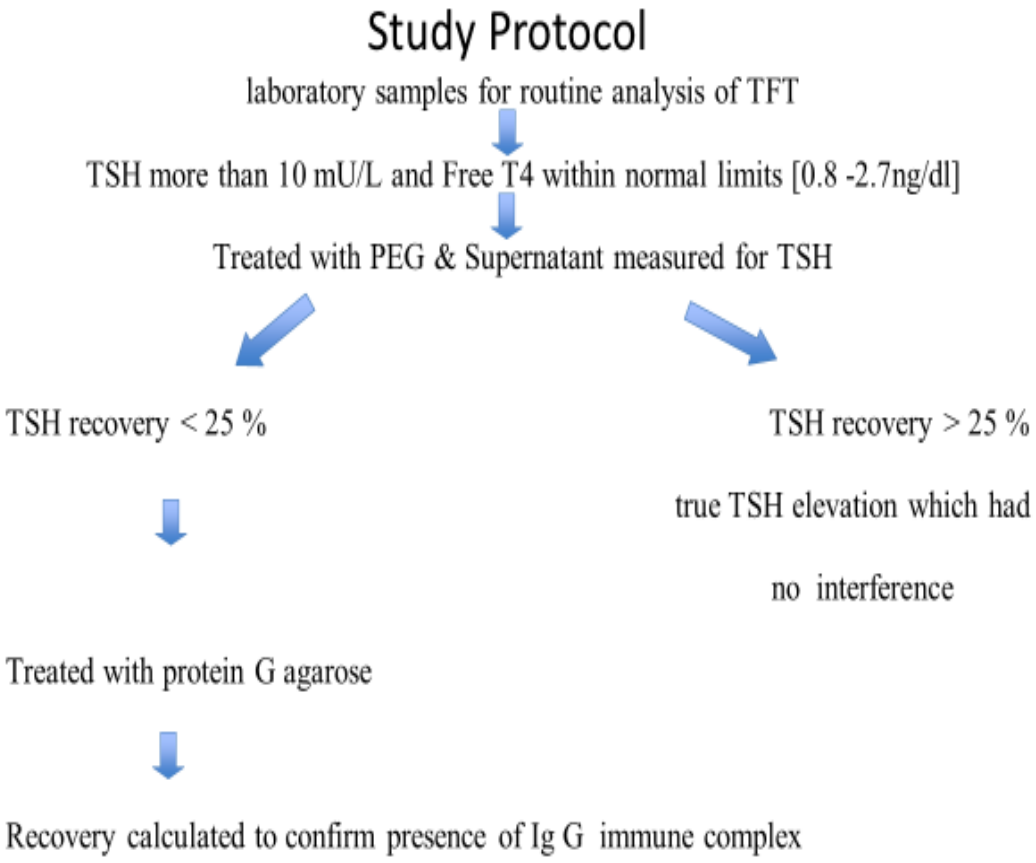
Known case of rheumatoid arthritis, chronic infection ,multiple myeloma and other malignancies.

SAMPLE SIZE

200.

STUDY PROCEDURE

- samples from patients subjected to routine analysis of thyroid function were scrutinised for TSH.
- Patients with TSH more than 10 mU/L and Free T4 within normal limits [0.8 -2.7ng/dl] were selected .
- Informed consent was obtained from 200 patients.
- Two aliquots of the each serum sample were taken.
- One aliquot of the patient ‘s serum was subjected to PEG precipitation test and other aliquot for protein G addition test.
- Samples which had > 25% recovery was treated as true TSH elevation which had no significant interference
- Samples which had a recovery of < 25% by PEG precipitation test were further subjected to protein G addition test.
- Samples that showed remarkable decrease in TSH recovery after protein G addition test suggested immunoglobulin G complex form of TSH.



METHOD

Sample collection and preparation

After getting informed consent from the patient, blood sample were collected under strict aseptic precautions in plain red topped venipuncture tubes without any additives or gel barrier. Allow the blood to clot. After centrifugation at 2000 -2500 rpm for 15 min, serum samples were separated immediately from the cells and analysed for TSH .

DETERMINATION OF SERUM TSH

REQUIREMENTS :

EQUIPMENT

- Cobas e 411/601 Analyzer
- Centrifuge
- vortex mixer.

METHOD

Electro-chemiluminescence immunoassay (ECLIA) for in vitro quantitative determination of free thyroxine, and thyroid stimulating hormone in human serum were performed using Automated Cobas e411 Immunoassay Analyzers based on electrochemiluminescent technology using ruthenium complex and the measuring cell.

'Electro' means electrical stimulation 'chemi ' refers to chemical reaction and luminescence indicate production of light.

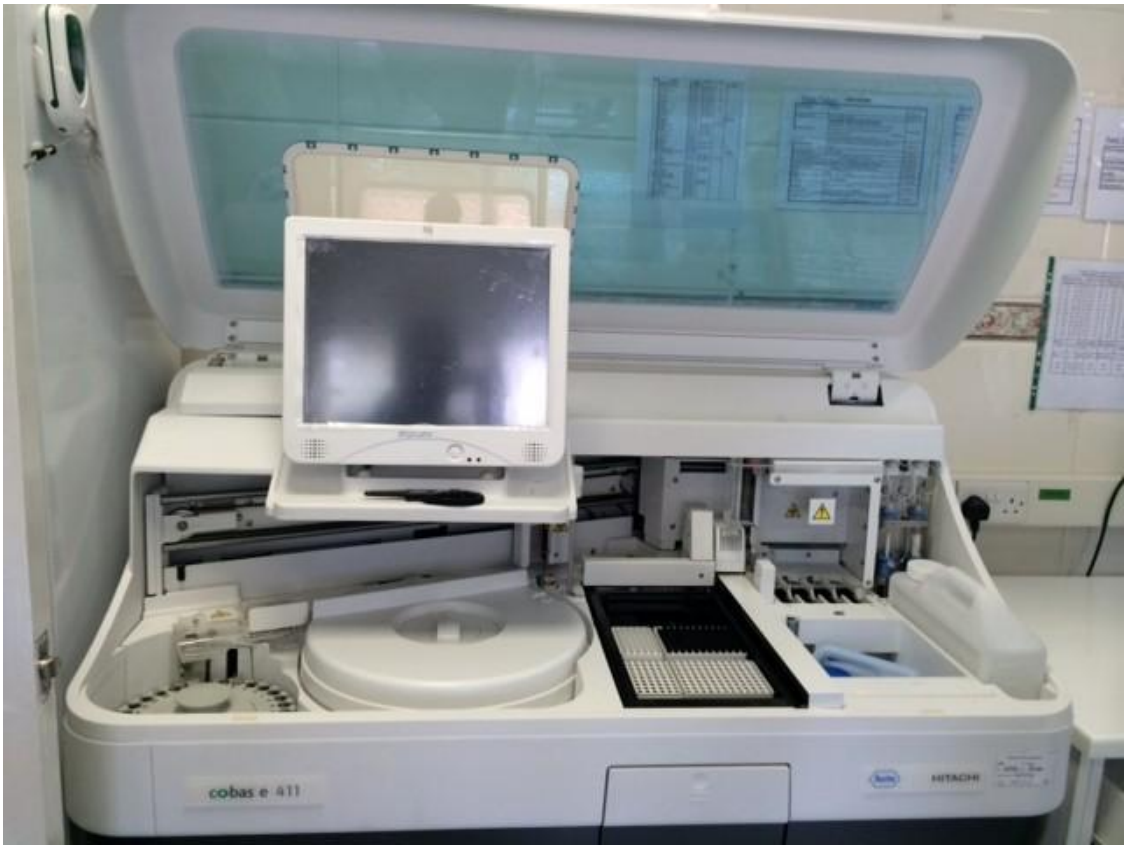


FIG 13: COBAS e 411 - ANALYSER

PRINCIPLE

- The biotinylated monoclonal antibody, antigen of interest, and monclonal antibody labeled with ruthenium complex form a sandwich complex
- After adding streptavidin-coated microparticles this complex binds to solid phase by interacting of biotin and streptavidin.
- Microparticles are magnetically captured onto the surface of the electrode

- . Application of a voltage to the electrode induces chemiluminescent emission which can be detected by a photomultiplier.

TEST PROCEDURE TSH IN ECLIA

Sandwich principle. Total duration of assay: 18 minutes.

1. **First incubation:[9 mins**]

50 uL of sample reacts with first biotinylated monoclonal TSH-specific antibody and then with second antibody monoclonal TSH-specific antibody labelled with a ruthenium complex. The two antibodies form a sandwich complex with the antigen or analyte of interest.

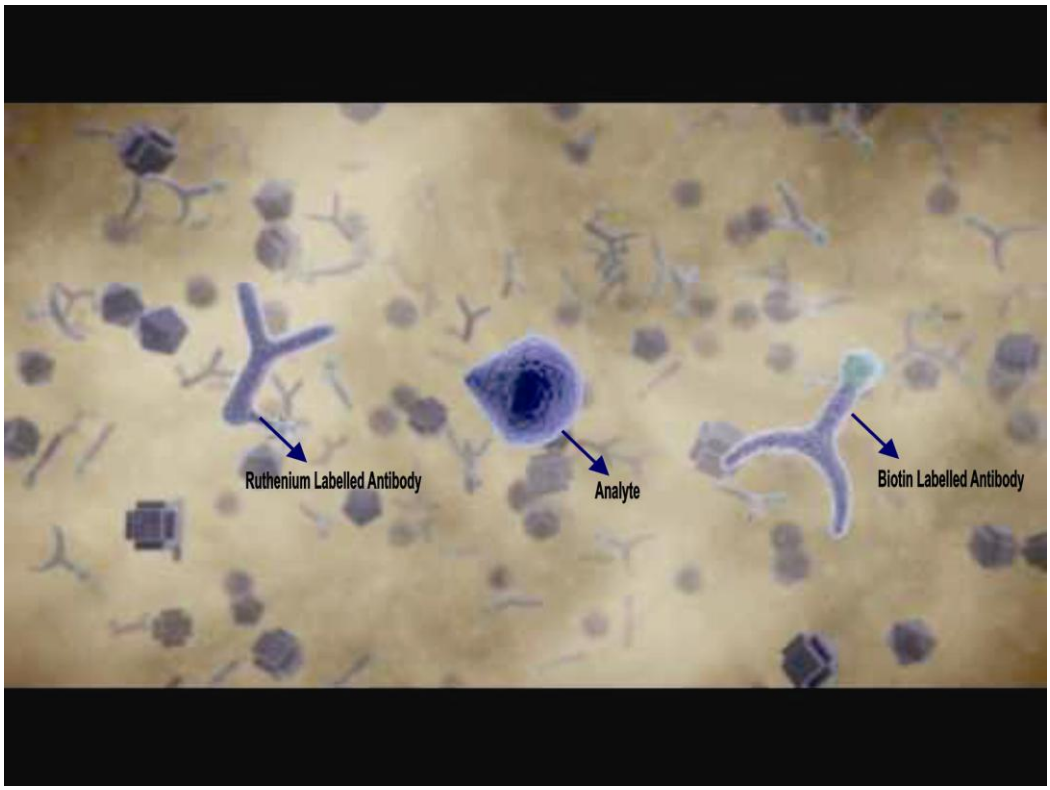


FIG 14: FIRST INCUBATION

2. Second incubation:[9 mins]

After addition of streptavidin-coated microparticles, the streptavidin forms a strong bond with biotin .

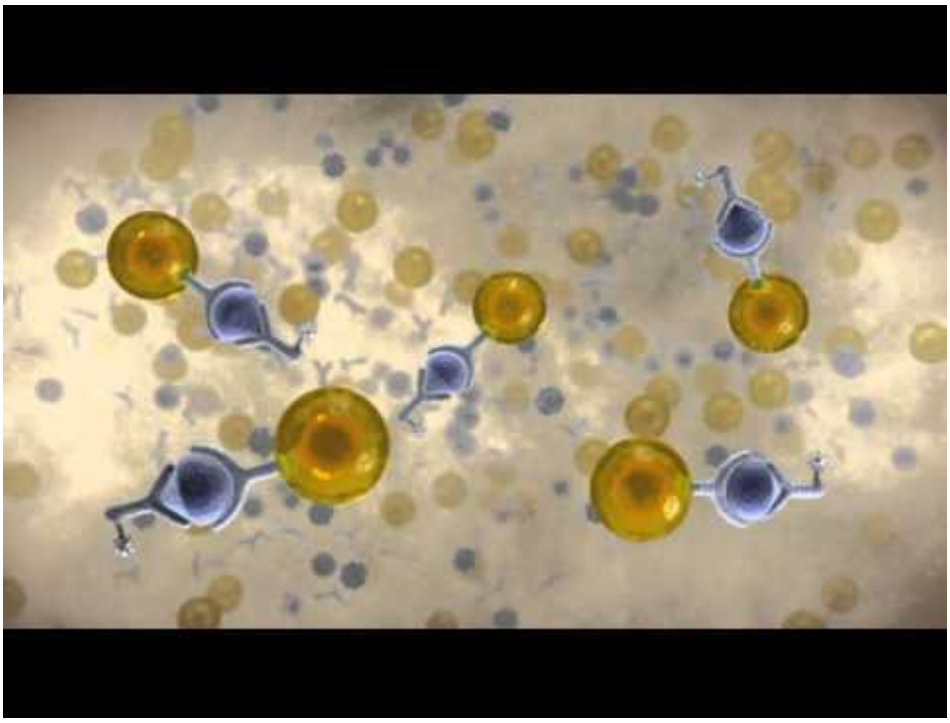


FIG 15: The sandwich complex formed .

3. This complete immunoassay complex is now transferred to measuring cell. The magnet present here attracts the paramagnetic beads , thus binding the complex to the surface of the measuring cell.

4. Now the procell applied ,which separates the particles bound to immunocomplex from the free particles. The procell also provides tripropylamine[TPA] which is essential for the electrochemi - luminescence [ECL] reaction to take place.

5. Electrodes present provides voltage which triggers the ECL reaction . Ruthenium and TPA gets excited.TPA serves as reductant. TPA enable ruthenium to reduce to its base state with release of light.

6. This cycle of electrochemiluminescence repeats as long as voltage is applied ,this results in amplification of light signals. The emitted light is detected by photomultiplier. The signal detected is directly proportional solution is to the concentration of the target analyte.

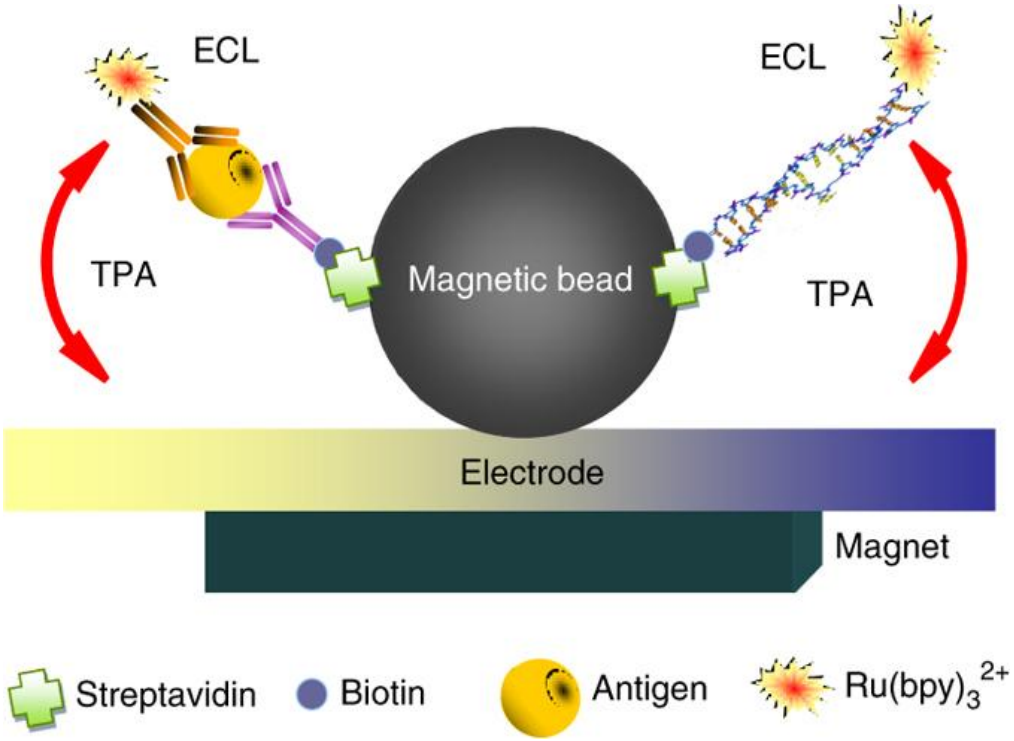


FIG 16: THE MAGNET ATTRACTS THE PARAMAGNETIC BEADS THUS BINDING THE COMPLEX.

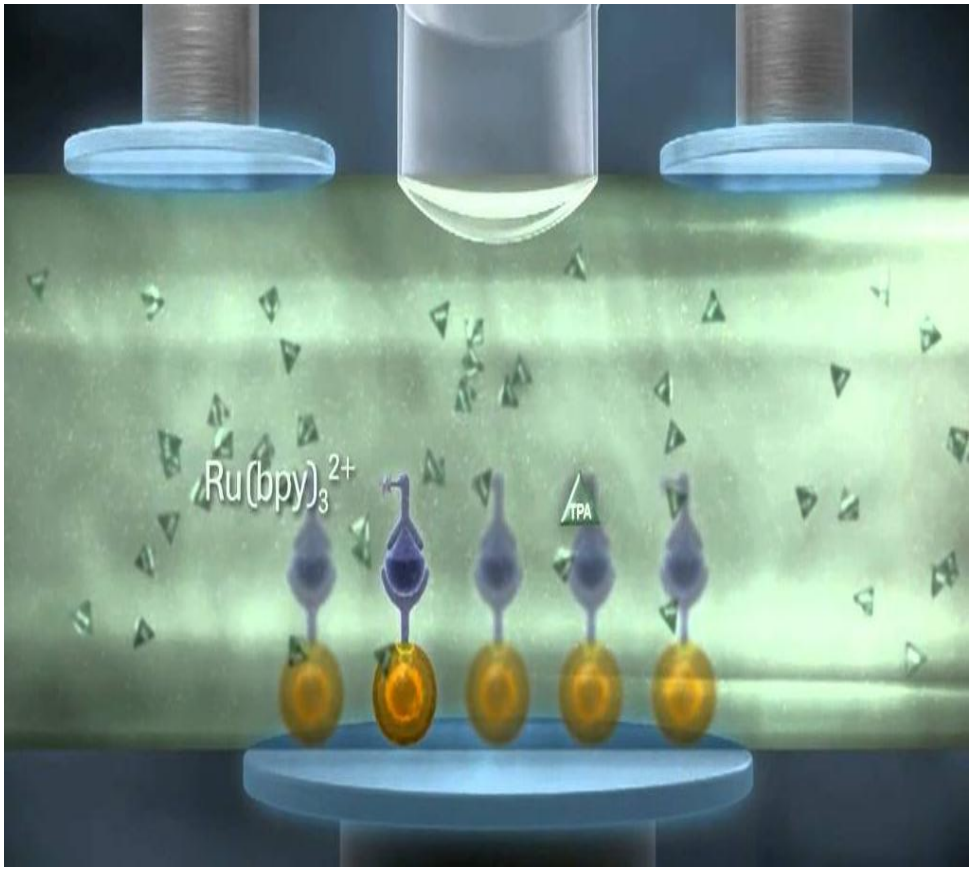


FIG 17 : TPA ENABLE RUTHENIUM TO REDUCE TO ITS BASE STATE WITH RELEASE OF LIGHT.

7. Results are determined with the help of a calibration curve which is instrument- specifically generated by 2- point calibration and a master curve provided by the reagent barcode.

REAGENT

TSH Elecsys from Roche Diagnostics

STORAGE AND STABILITY

Store at 2-8 °C. Do not freeze.

MEASURING RANGE

0.005-100 µIU/mL .

Values below the lower detection limit are reported as < 0.005 µIU/mL. Values above the measuring range are reported as > 100 µIU/mL (or up to 1000 µIU/mL for 10-fold diluted samples)

Functional sensitivity - 0.014 µIU/mL

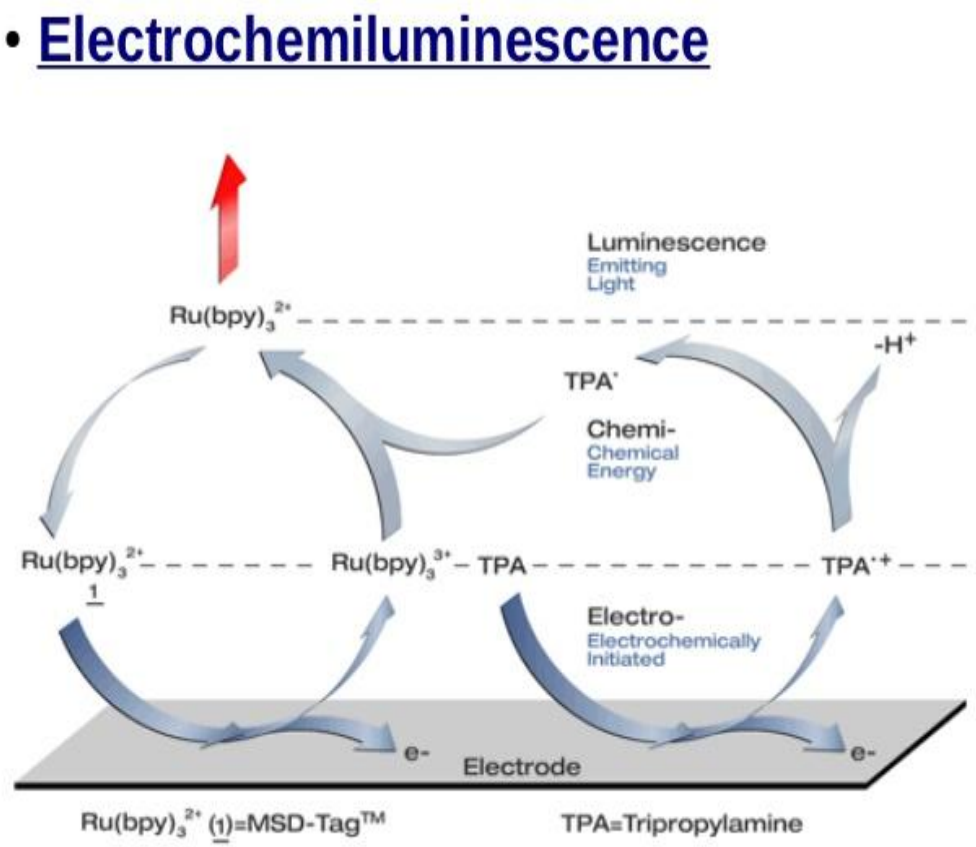


FIG 18: Ru(bpy)3^{2+} COMPLEX – TPA (TRIPROPYLAMINE) FORMS A REDOX COUPLE EMITTING LIGHT SIGNALS

FREE T4 ASSAY

Principle – Competitive assay

Total duration of assay: 18 minutes.

• 1st incubation: 15 µL of sample and a T4-specific antibody labelled with a ruthenium complex.[9 min]

• 2nd incubation: After addition of biotinylated T4 and streptavidin-coated microparticles, the still free binding sites of the labelled antibody become occupied, with formation of an antibody-hapten complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.[9 min]

• The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with Procell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

• Results are determined via a calibration curve which is instrument-specifically generated by 2point calibration and a master curve provided via the reagent barcode.

REAGENT

Free T4 from Roche Diagnostics

STORAGE AND STABILITY

Stability

Unopened at 2 to 8 °C – upto stated expiry date

After opening at 2 to 8 °C – 12 weeks

On the analyser – 8 weeks

Store at 2 to 8 °C

Do not freeze.

Store the Elecsys reagent kit upright in order to ensure complete availability of the microparticles during automatic mixing prior to use.

LIMITS AND RANGES

RANGE	FREE T4	TSH
MEASURING RANGE	0.101-7.77 ng/Dl	0.005-100.0 mlU/L
REFERENCE RANGE	0.8 -2.7ng/dl	0.4 - 4.2 mIU/L

Measuring range for FT4

0.101-7.77 ng/dL

Values below the Limit of Quantitation are reported as < 0.101 ng/dL

Values above the measuring range are reported as > 7.77 ng/dL .

Functional sensitivity = 0.101 ng/dL

REFERENCE RANGE: TSH

 Child

Birth to 4 days: 1 - 39 mIU/ml

 Adults : 0.4 - 4.2 mIU/ml

 Pregnancy

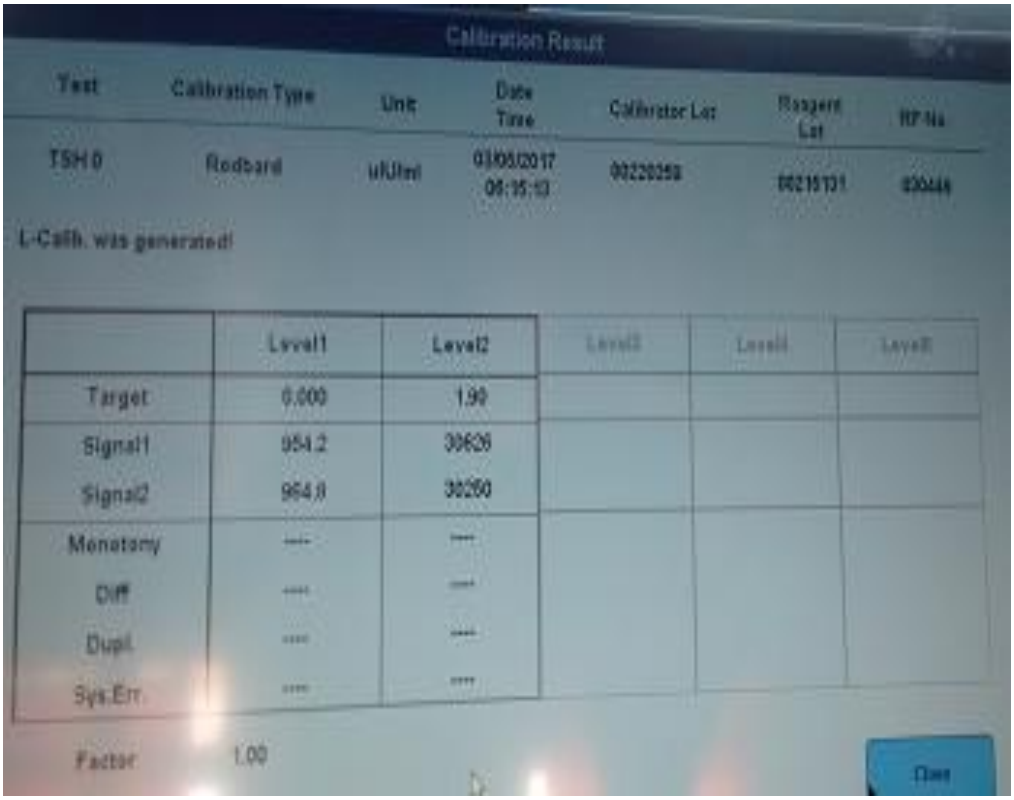
I trimester : 0.3 - 4.5 mIU/ml

II trimester : 0.5 - 4.6 mIU/ml

III trimester : 0.8 - 5.2 mIU/ml

CALIBRATION

Calibration performed once per reagent lot using fresh reagent . Two set of two levels of calibrators CAL 1 and CAL 2 are provided in separate kit for each analyte.



L – CALIBRATION WAS GENERATED.

QUALITY CONTROL MATERIAL

QC given

CENTRIFUGE MINI SPIN.[Eppendorf]

- Mini centrifuge was used for separation of serum after PEG precipitation and protein G addition test
- the main switch is turned on and lid is opened
- rotor was loaded symmetrically
- for PEG precipitation test mixture was centrifuged for 5min at 13,400 rotations per minute
- for protein G addition test the mixture was centrifuged at 12000 g for 5min

POLYETHYLENE GLYCOL[PEG] PRECIPITATION METHOD

TSH values of more than 10 mU/L and FT4 within normal limits are evaluated by PEG precipitation method.

PRINCIPLE

PEG precipitation method is a simple procedure for precipitating immunoglobulins G, A and M from the serum. Precipitation of PEG is based on polymer effect and steric exclusion effect.

$$V_T = V' + V_E$$

- V_T - Assuming total volume of solvent occupied by polymer and protein

- V_E – volume occupied by polymer(excluded volume not occupied by protein)

- V' – volume occupied by protein

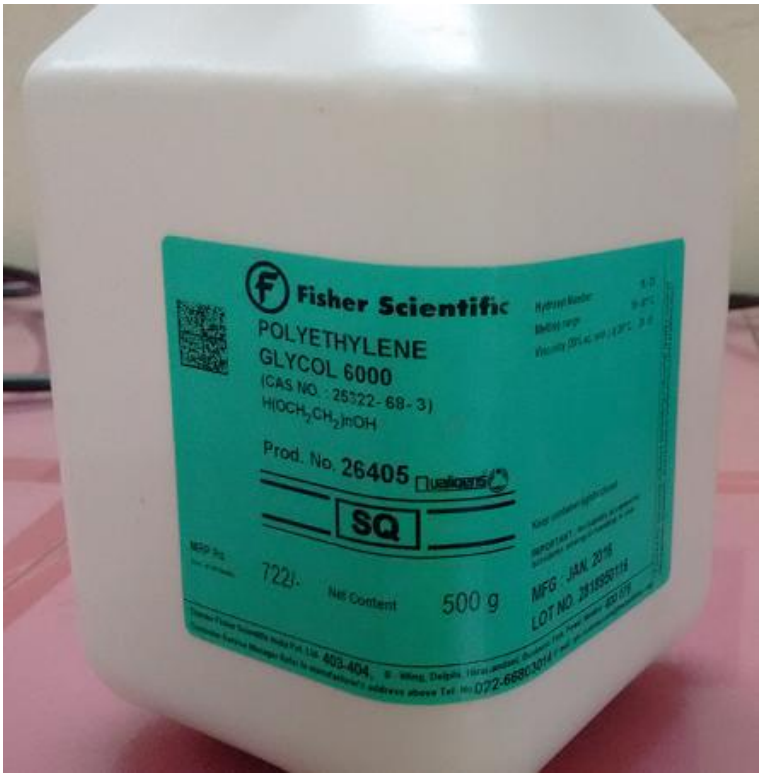


FIG 19: POLYETHYLENE GYCOL 6000

PROCEDURE

PEG precipitation was used as a simple method of precipitating immunoglobulins G, A and M [5].

1. A 25% solution of PEG 6000 was prepared in deionised water.
2. A 0.2mL of serum aliquot was added to 0.2mL of the 25% PEG 6000 solution (TSH.PEG).
3. To 0.2mL of deionized water, 0.2mL of the 25% PEG 6000 solution was added (TSH.H2O) as a control.
4. The samples were vortex mixed and incubated for 10min at room temperature.
5. Then the mixture was centrifuged for 5min at 13,400 rotations per minute.
6. The TSH concentration of the supernatant was assayed using cobase e 411.

FIG 20: PEG PRECIPITATION TEST – The supernatant and precipitate can be observed.

8.The percentage recovery of TSH following PEG precipitation was calculated .

TEST	PEG	SERUM	DEIONISED WATER
CONTROL	0.2mL	_	0.2Ml
SAMPLE	0.2mL	0.2Ml	_

CALCULATION:

$$Recovery\ \%of\ TSH = \frac{TSH.PEG}{TSH.water} * 100$$

PROTEIN G ADDITION TEST

The samples with recovery of less than 25 % were further treated with protein G addition test for confirming the presence of IgG complex TSH. Samples showing remarkable decrease in protein G addition test suggested immunoglobulin G form of TSH.[7]

PRINCIPLE

Protein G agarose gel absorbs Ig G complexed form of TSH. Therefore the macroTSH which mainly occurs in the form Ig G complexed TSH are absorbed by protein G addition test.

PROCEDURE

1. Protein G agarose was used for absorption of serum Ig G.
2. A 0.2mL of serum aliquot was added to 0.2mL of the protein G agarose and incubated at 4 ºC for 3 hr.
3. The above mixture was centrifuged at 12000 g for 5min

4. The supernatant was measured by elecsys for TSH value.

5. Recovery calculated.

6. Samples showing remarkable decrease in protein G addition test suggested immunoglobulin G complexed form of TSH.

CALCULATION :

$$Recovery\ \%of\ TSH = \frac{TSH.IgG}{TSH.water} * 100$$

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STATISTICAL ANALYSIS & RESULTS

RESULTS

STATISTICAL ANALYSIS AND RESULTS

In this study, 200 serum samples were collected with TSH value more than 10 µIU /mL and FT4 within normal range [0.8 -2.7ng/dl]. First aliquots of all 200 samples were treated with PEG and second aliquots of serum samples with recovery less than 25% were further subjected to protein G addition test. Of 200 sample 9 sample had a post PEG recovery of less than 25% that is 4.5% of the total sample collected. After subjecting 9 samples to protein G addition test 4 serum samples showed a marked decrease in recovery percentage suggesting 2% of samples had macro TSH .

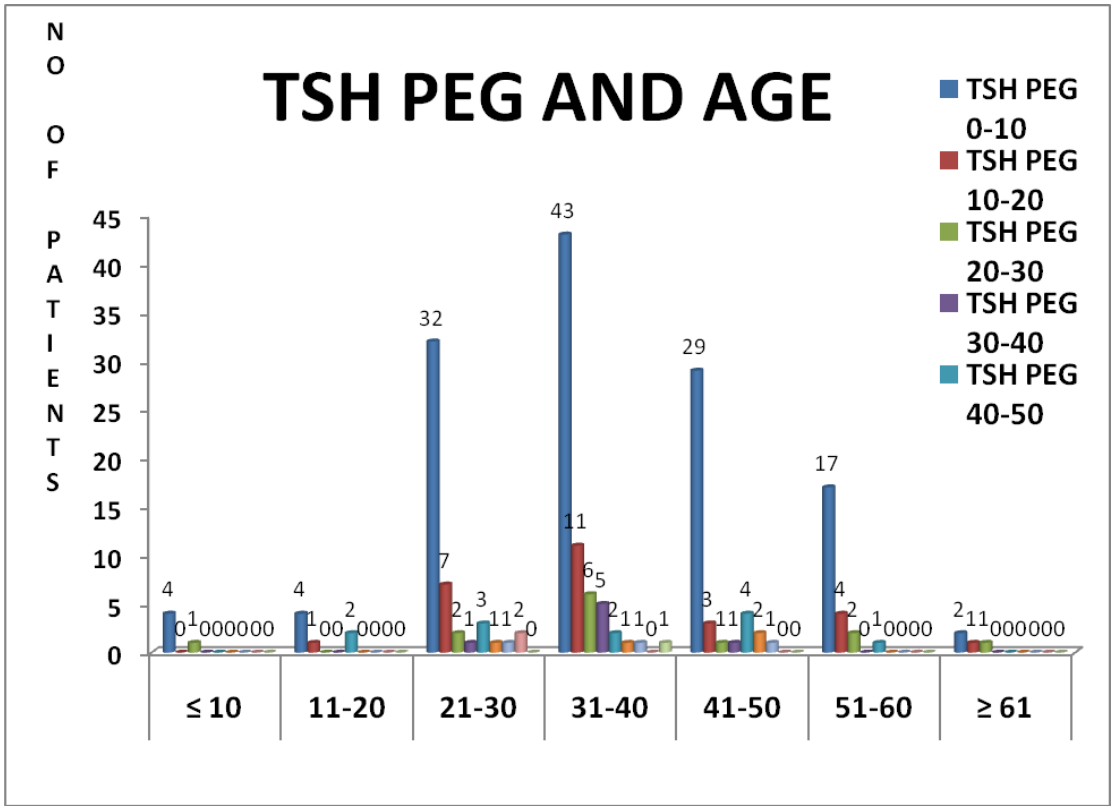
Results of laboratory values obtained from PEG treated TSH were compared with the controls values ie ,TSH treated with deionized water and were statistical analysis using excel software. Student's paired 't' test was used to compare the means between two variables . Pearson coefficient of correlation was used to estimate the degree of association between PEG precipitation method and protein G addition test . A p-value of <0.05 considered as statistically significant

Table-1

TABLE SHOWING GENDER DISTRIBUTION OF SERUM SAMPLES COLLECTED.

Gender	**NUMBER (N)**	**PERCENTAGE (%)**
MALE	22	11.00
FEMALE	178	89.00
TOTAL	**200**	**100**

GRAPH 1: SHOWING AGE DISTRIBUTION OF SERUM SAMPLES COLLECTED



This graph shows that most of the TSH samples collected were between 30 to 40 years of age.

Table-2

AGE DISTRIBUTION OF PATIENTS

AGE GROUP	MALE		FEMALE		TOTAL	
	NO	%	NO	%	NO	%
≤ 10	0	0	5	2.81	5	2.50
11 – 20	3	13.64	4	2.25	7	3.50
21 – 30	3	13.64	46	25.84	49	24.50
31 – 40	5	22.73	65	36.52	70	35.00

41 – 50	3	13.64	38	21.35	41	20.50
51 – 60	7	31.82	17	9.55	24	12.00
≥ 61	1	04.55	3	1.69	4	2.00
TOTAL	**22**	**100**	**178**	**100**	**200**	**100**

Table showing age and gender distribution among the sample collected.

Table-3

TABLE SHOWING THE RESULT AFTER PEG PRECIPITAION TEST AND PROTEIN G ADDITION TEST.

VARIABLES N=200	RECOVERY PERCENTAGE	NO OF SAMPLES	PERCENTAGE OF SAMPLES
PEG PRECIPITATION	>25 %	191	95.5%

TEST	< 25%	9	4.5%
PROTEIN G ADDITION TEST	< 31%	4	2%

This table shows that among 200 samples treated with PEG, 95.5% sample has a recovery of more than 25% and 4.5% of samples have a recovery of less than 25%. After protein G addition test 2% of the sample shows results suggestive of macro TSH.

Table-4

SCORES COMPARATIVE OF VARIABLES

VARIABLES	**MALE**		**FEMALE**		**TOTAL**	
	MEAN	**SD**	**MEAN**	**SD**	**MEAN**	**SD**
TSH	34.40	31.41	36.95	30.91	36.67	30.90

FT4	0.94	0.17	0.97	0.23	0.97	0.22
TSH .PEG	11.08	11.93	14.12	17.48	13.78	16.96
TSH .WATER	17.66	17.81	21.02	22.25	20.65	21.79
RECOVERY%	63.04	18.96	62.85	19.40	62.87	19.30

Table showing mean and SD (standard deviation) of variables analyzed in the study.

Table-5

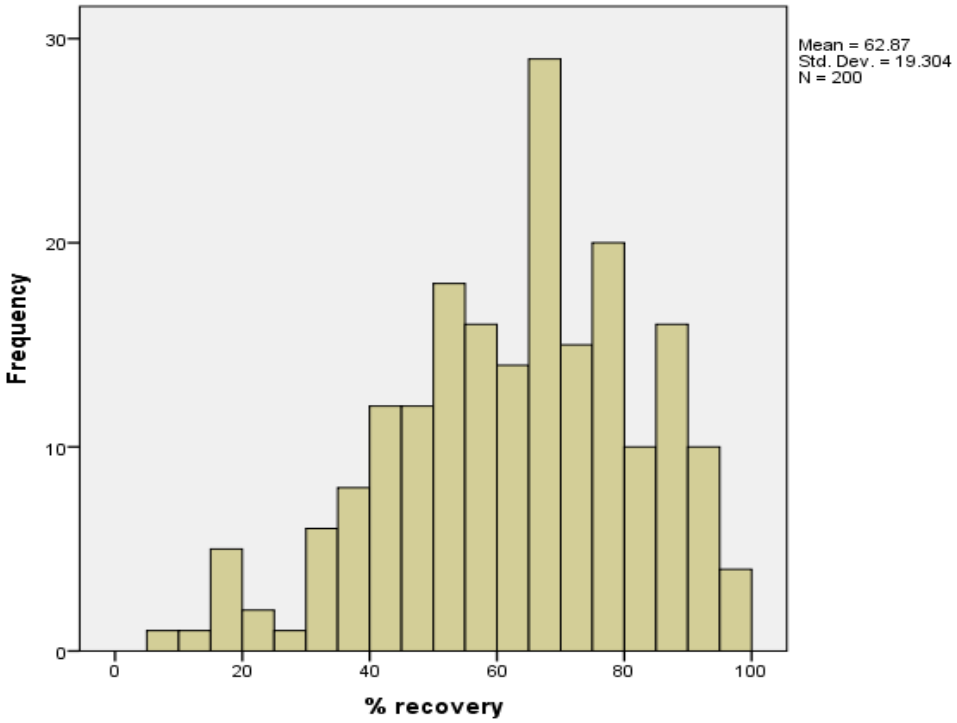
PAIRED SAMPLE T-TEST (Pre Vs Post PEG treated values)

VARIABLE **N=200**	**MEAN**	**SD**	**t-value**	**p-value**	**Significant**

TSH .PEG	13.78	16.96	12.84	0.0001	Significant
TSH.WATER	20.65	21.79			

TSH treated with deionized water [TSH .WATER] is taken as control vs TSH treated with PEG and the values are compared by paired t test and p – value was derived. The test was significant.

Graph.2 Histogram of percentage recovery of TSH after polyethylene glycol precipitation



RECOVERY PERCENTAGE AFTER PEG TREATMENT FOR 200 SAMPLES SHOWS A MEAN OF 62.87 AND SD OF 19.3

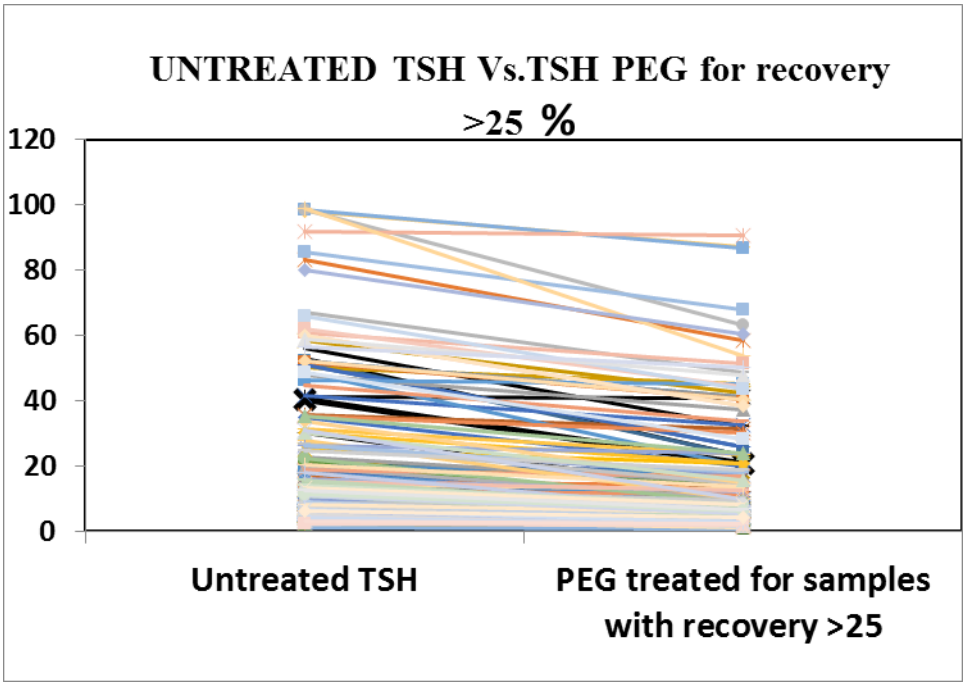
Table-6

COMPARING RECOVERY PERCENTAGE AFTER PEG PRECIPITATION TEST AND RECOVERY PERCENTAGE AFTER PROTEIN G ADDITION TEST

TSH	N	MEAN	SD	t-value	p-value	Significant
PEG %	9	17.09	3.90	3.19	0.01	Significant
Ig G %	9	30.67	12.18			

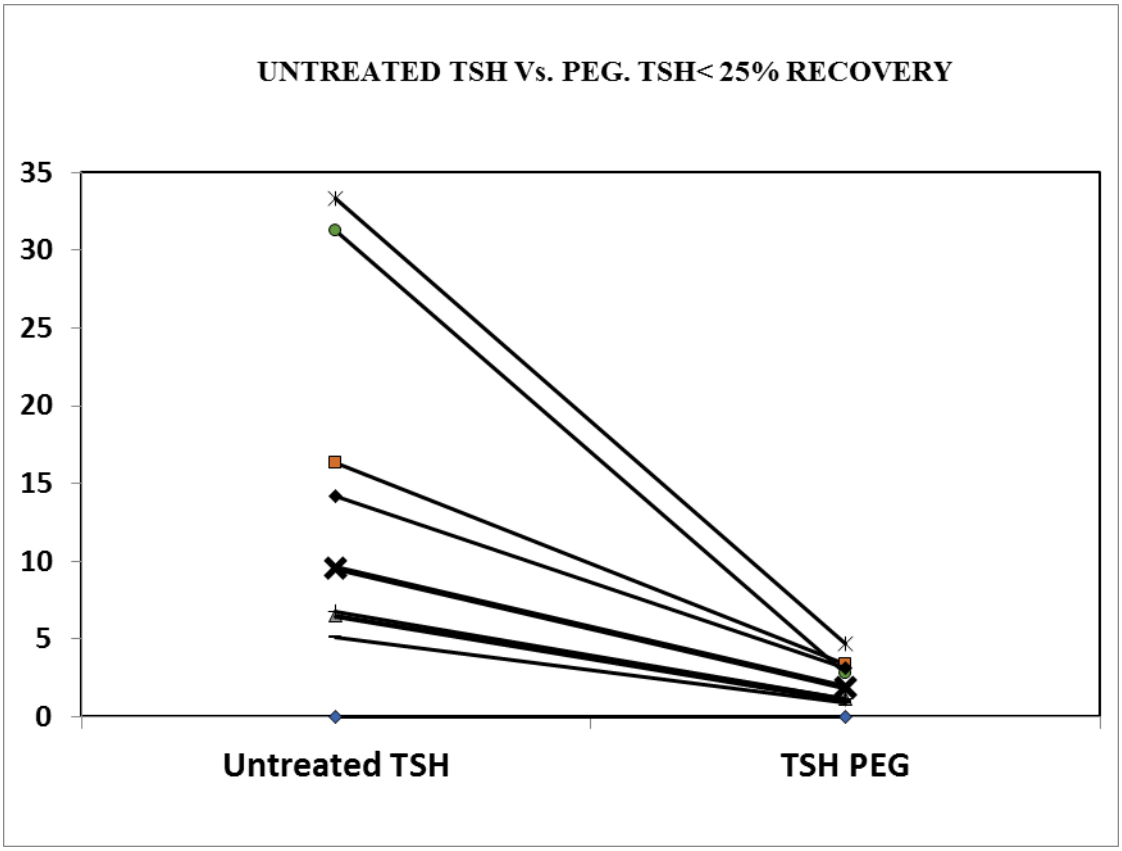
Serum sample with recovery < 25% in PEG precipitation method compared with recovery percentage after subjecting them to protein G addition test. The test was significant .

GRAPH 3: COMPARING UNTREATED TSH AND PEG TREATED TSH > 25% RECOVERY



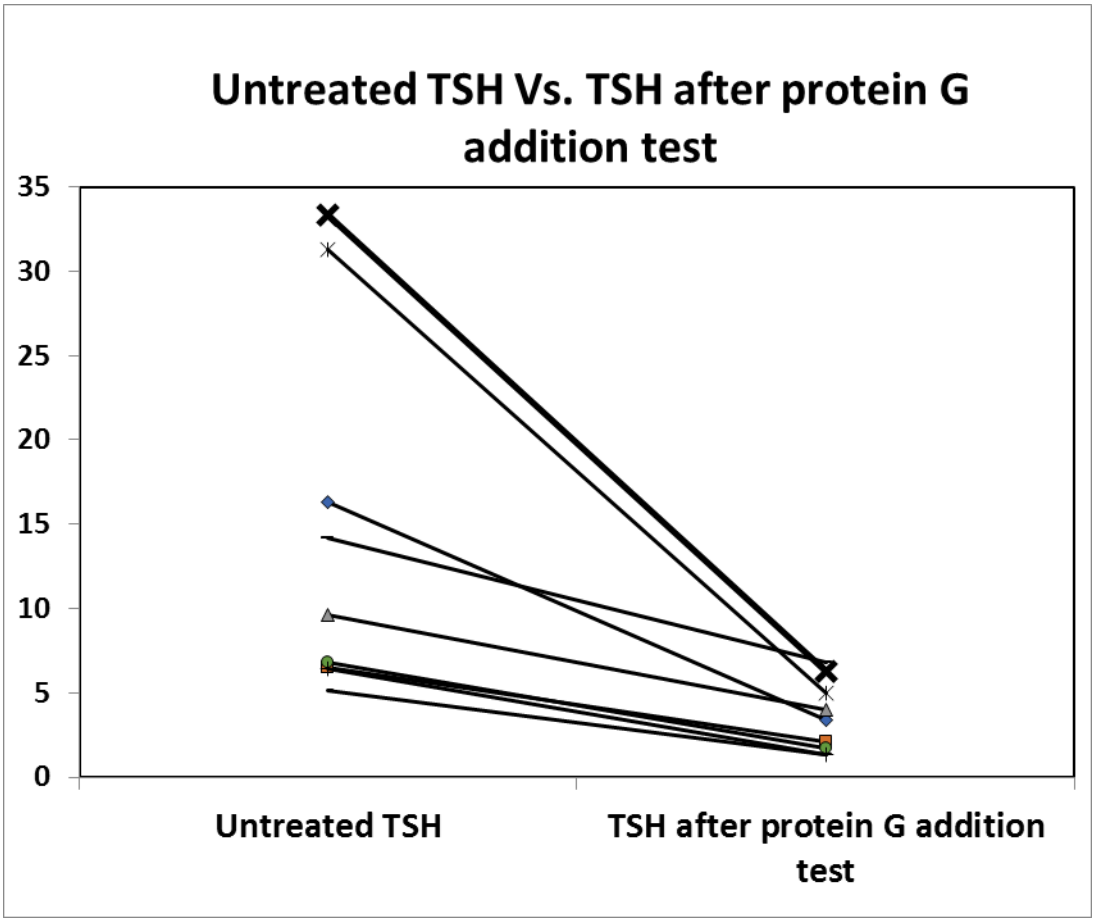
GRAPH 3 : Shows serum TSH concentrations before and after serum treatment with PEG in patients with true TSH elevation [ie] for recovery > 25 %. Of 200 samples 191 samples had PEG recovery more than 25%. Comparing untreated TSH with PEG treated TSH samples there is no marked decrease in value showing no interference due to macro TSH.

GRAPH 4 : COMPARING UNTREATED TSH AND POST PEG TREATED TSH < 25% RECOVERY



9 [4.5%] out of 200 samples showed a recovery of less than 25% with PEG. A drastic decrease in TSH values after PEG treatment is noted.

GRAPH 5 : COMPARING UNTREATED TSH AND AFTER PROTEIN G ADDITION TEST .



GRAPH 4: Serum TSH concentrations before and after protein G addition test. 4 [2%] out of 9 samples shows marked decrease in TSH values after the test showing a recovery of less than 31 % suggesting macroTSH.

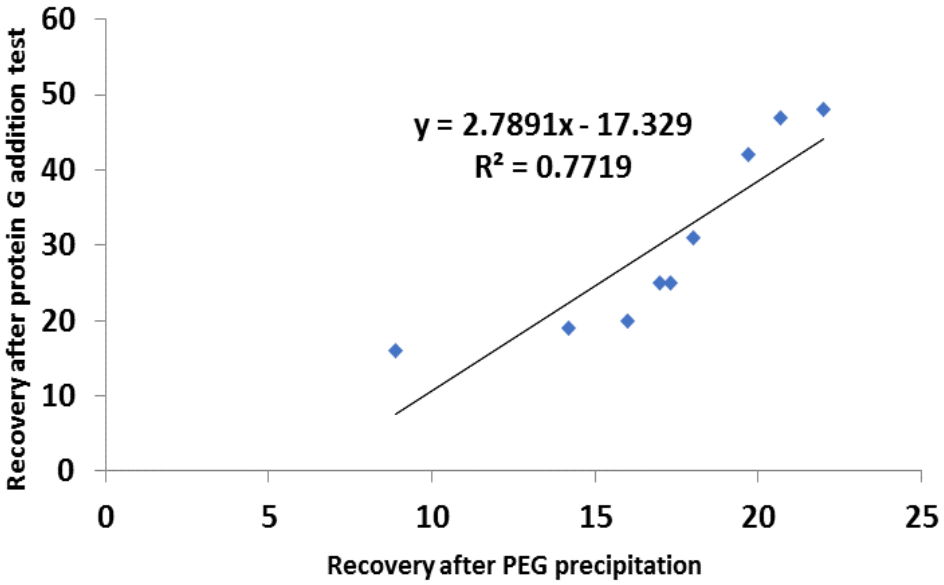
Table-6

CORRELATION BETWEEN PEG RECOVERY AND PROTEIN G RECOVERY .

	r-value	**p-value**	**Significant**
PEG RECOVERY	0.89	0.01	Significant
Vs			
PROTEIN G RECOVERY .			

Table 6: Explains the Pearson's Correlation Coefficient Between Biochemical Parameter of PEG precipitation test and protein G addition test. It Revealed Positive Correlation Between both the recoveries

GRAPH 6 : REGRESSION ANALYSIS BETWEEN PEG PRECIPITATION AND PROTEIN G ADDITION TEST



GRAPH 6: explains the correlation of recovery percentage after PEG precipitation and protein G addition test . PEG has a positive correlation with protein G addition test as indicated by the upward slope of linear regression analysis and the r value is equal to 0.89.

DISCUSSION

DISCUSSION

Endogenous antibodies are one of the commonest cause of interference in TSH assay. One of the study showed that out of 5130 sets of results, 28 (0.53%) of them were analytically incorrect results [5]. Out of which 23 patients had a potential adverse effect on the expense and clinical care. There are several causes of interference in thyroid hormone assay, of which some of them have been virtually eliminated from modern assays while other causes of interference like drugs, antibodies and macrocomplexes still persist. The clinical consequence of these interferences may lead to false elevation improper therapeutic decision and unnecessary follow up examination.

True hypothyroidism is characterised by presence of excess of monomeric form of TSH found in serum but if macroTSH is present along with monomeric form it can give rise to false elevation of TSH. In subclinical hypothyroidism, TSH value more than 10 µIU /mL is taken as a cut off for initiating thyroid replacement therapy [48,49]. In order to avoid misdiagnosis of subclinical hypothyroidism the possibility of macro TSH should be considered in elevated TSH values [7] . Macro TSH which is considered to be biologically inert may not require thyroid replacement therapy and the patients may be

subjected to unnecessary treatment which may cause mismanagement[75]. In the study published by Hiroyuki sakai et al [7] elevated thyroid-stimulating hormone (TSH) was also found during regular neonatal screening in a newborn with no clinical symptoms. Thyroid function tests were repeated and confirmed to have high TSH value but normal total thyroxine (T4) and triiodothyronine (T3). The mothers of the neonates also had elevated serum TSH with normal levels of T4 and T3. This suggested a transmitted maternal interfering factor, and no treatment was started while further investigation suggested that serum from both the infant and the mother showed a peak TSH with molecular mass consistent with a TSH-IgG complex (macro-TSH) [77]. The TSH values in the infant also declined to a normal level within 8 months in pace with a normal rate of elimination of maternal IgG .These cases suggests that interfering macro-TSH should also be considered in a euthyroid neonate with elevated serum TSH and normal T4 and T3 levels to avoid unnecessary treatment.

PEG precipitation is commonly used to detect the interference of macro prolactin in clinics, but it is also used in cases that are suspected to have interference of TSH. PEG precipitation is a simple and cost effective technique which can be used as screening test for immunoglobulin complexed TSH [macro TSH]. PEG is also known to precipitate a portion of free analyte along with immunoglobulin, so it is necessary to determine an analyte and method specific reference range for PEG precipitation method. According to study conducted by Francesca Mills et al., a cut off of < 25 % can be used as

recovery percentage for TSH after PEG precipitation. This cut off can be taken for directing the samples which may need further investigation into the possibility of presence of macroTSH. PEG precipitation is relatively crude and subjected to certain limitation. The presence of increased globulin concentration may cause an increase in the amount of monomeric hormone precipitation with PEG. Other limitation of PEG is that it can interfere with some immunoassays.

In this study 200 hypothyroid cases were analysed before and after PEG treatment .TSH values of PEG untreated sera ranges from 10.1 to 99.2 with mean 36.67 and SD of 30.90. After the 200 serum were treated with PEG, the TSH was reanalysed which showed a decrease in TSH values of all sera. The TSH values of PEG treated samples ranges from 0.69 to 90.5 with mean of 13.78 and SD 16.96. TSH treated with deionised water used as a control ranges from 1.28 to 98.7 with mean of 20.65 and SD of 21.79.

The present study showed that among 200 serum samples collected, with TSH more than 10 mU/L and FT4 within normal limits, 9 [4.5%] exhibited PEG-precipitable TSH ratios less than 25% recovery. This finding was consistent with the study done by Francesca Mills et al, which showed a similar result. Out of 9 serum sample, 4 [2%] of them showed a remarkable decrease in TSH recovery after protein G addition test .

In the study conducted by Francesca Mills et al., [5] low recovery percentage after PEG precipitation test < 25 % was detected in 15 (3 %) out of 495 serum sample with TSH measurement more than 10 mU/L and macro TSH were identified in 3 of 15 serum samples by gel filtration chromatography. Gel filtration chromatography test is the suggested test for the diagnosis of macro-TSH. But the test could not be performed because of the cumbersome procedure and cost. However, gel filtration chromatography may sometime do not differentiate the causes of interference [76] due to cross linking mechanism [eg heterophile antibodies] . Hence additional work to prove macroTSH like preincubation with urea, using acid elution buffer or selective precipitation with protein A or G sepharose is also proposed.

Hiroyuki sakai et al [7] suggested protein G addition test for identification of macro TSH. In study conducted by Hiroyuki sakai et al [7] sample with recovery of 31% by protein G addition test suggested the presence of macroTSH. In the present study 4 patients showed a remarkable decrease in recovery % with protein G addition test in consistence with the study performed by hiroyuki sakai et al. The remarkable decrease in TSH recovery after protein G addition test suggested that most of the TSH complex were removed by protein G agarose gel which mainly absorbs Ig G. Therefore most of the TSH existed in the form of macro TSH which is an immune complex of anti -TSH auto antibody mostly in the form of IgG and TSH. [7]

SUMMARY

SUMMARY

The study of evaluation of immunoglobulin G complexed form of thyroid stimulating hormone [macro TSH] as interference in TSH assay was conducted in our tertiary care hospital.

200 serum samples were collected with TSH value more than 10 µIU /mL and FT4 within normal range [0.8 -2.7ng/dl]. Each serum sample were taken in two aliquots. Of all 200 samples were treated with PEG , serum samples with recovery less than 25% recovery were further subjected to protein G addition test.

Among 200 serum samples, with TSH more than 10 mU/L and FT4 within normal limits, 9 samples exhibited PEG-precipitable TSH ratios less than 25% recovery and were subjected to protein G addition test . Out of 9 serum sample 4 [2%] sample showed remarkable decrease in TSH recovery after protein G addition test suggesting most of the TSH in those samples exist in the form of macro TSH which were mostly IgG bound.

To conclude it is important that clinical biochemists are aware of possible causes of immunoglobulin interference in TSH assays, including the

presence of macro-TSH, to prevent inappropriate interpretation of thyroid function test results from influencing clinical care.

CONCLUSION

CONCLUSION

From the result of our study, we conclude that, Macro TSH mostly occurs in the form of TSH complexed with IgG . 9 (4.5%) serum samples with recovery less than 25 % with PEG were further subjected to protein G addition test 4 (2%) samples with remarkable decrease in TSH recovery after protein G addition test suggesting to have macro TSH. The clinician and laboratory personnel should be aware of macro TSH as one of the cause of false elevation of TSH. Macro TSH should be suspected as one of the cause of interference when there is discordance between TSH level and the clinical features of the patient. Because macro TSH exhibited low bioactivity, thyroid hormone replacement therapy may not be required in patients with macro TSH. PEG precipitation, which is a simple and cheap method, can be used as a screening test and samples with recovery less than 25 % should be confirmed further. Gel filtration chromatography is gold standard method for confirmation of macro TSH. As it is expensive , cumbersome for routine analysis and also fails to differentiate interferences [5] caused by cross linking mechanism [eg heterophile antibodies] , protein G addition test was used.

FUTURE PERSPECTIVE:

The findings of this study are only suggestive. A larger sample size and further confirmation of serum samples with gel filtration chromatography can be done to arrive at definite conclusion.

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MASTER CHART

s.no	age	sex	TSH µIU/mL	FT4 ng/dl	TSH.PEG	TSH.H2O	% PEG recovery	IgG .TSH	IgG recovery%
1	35	F	14.5	1.1	2.5	7.1	35.3		
2	34	F	16.3	1.3	3.08	8.2	37.5		
3	55	F	13.8	1.1	4.16	6.1	67.2		
4	53	F	90.7	0.8	20.7	40.3	51		
5	50	F	39.1	1.2	41	41.3	99		
6	22	F	14.2	1.2	5.5	7.2	76		
7	35	F	62.3	0.8	12.1	30.2	40		
8	39	F	95	0.8	32.1	56	57		
9	31	M	96	0.8	23	53	43		
10	40	F	11.4	1	1.5	2.7	55		
11	28	F	15.9	1.1	3.1	6.7	46.2		
12	30	F	11.7	1.5	1.3	2.2	58		
13	49	f	14.5	1.1	2.2	5.3	41		
14	29	f	10.9	1.2	2.1	4.3	48		
15	33	F	33.7	1	8.2	10.5	78		
16	30	F	11.4	1.4	2.5	2.8	89		
17	29	F	98	0.8	23.1	52	44		
18	48	F	16.1	1.1	3.08	7.4	41.5		
19	23	F	15.9	1	6.1	7.2	84.7		

20	48	F	12.2	1.3	2.8	4.3	65.1		
21	40	F	18.5	1.1	5.8	9.3	62.3		
22	40	F	24.5	0.8	6.6	8.6	76.7		
23	48	F	10.6	0.9	3.7	5.2	71.1		
24	29	F	58.1	1.6	12.3	17.3	71.09		
25	37	F	31.6	1	3.4	16.3	**20.7**	7.6	47%
26	56	F	10.5	1	1.17	6.5	**18**	**2.1**	32%
27	40	f	12.6	1	4.1	6.2	66.1		
28	40	F	18.5	1.1	1.9	9.6	**19.7**	4.03	42%
29	30	F	14.5	0.9	5.2	7.2	72.2		
30	10	F	11.4	1	4.2	6.7	62.6		
31	27	F	53.6	0.9	16.4	21.1	77.7		
32	50	F	76.6	0.9	4.7	33.3	**14.2**	6.3	19%
33	35	F	94	0.8	25.8	51.1	50.4		
34	24	F	97	0.8	12.01	17.3	69.4		
35	29	F	12.5	0.9	4.1	6.2	66.1		
36	40	F	13.2	1.16	31.2	35.8	87.1		
37	25	F	15.4	1.08	4.1	6.1	67.2		
38	27	F	32.9	0.9	10.5	17.5	60		
39	26	F	16.8	1.1	3.1	7.1	43.6		
40	40	F	13.2	1.16	3.3	8.9	37.07		
41	57	F	43.1	0.8	18.3	20.3	90		
42	35	F	14.2	0.9	3.8	4.05	93.8		
43	34	F	17.6	1.01	5.1	7.8	65.3		
44	30	M	98	0.8	42.2	58.6	72.01		
45	51	M	30.6	1.6	6.2	15.7	39.4		
46	50	F	17.1	0.9	7.1	8.5	83.5		
47	27	F	27.2	0.8	10.5	18.5	56.7		

48	28	F	14.4	1.02	4.09	7.5	54.5		
49	48	F	13.1	0.9	2.6	5.5	47.2		
50	29	F	93	0.8	45.2	50.1	90.2		
51	5	F	15.5	2.4	3.5	5.3	66		
52	35	F	10.9	1.08	4.5	5.7	78.9		
53	27	F	27.2	0.8	7.12	10.4	68.4		
54	34	F	28.5	1	10.1	16.7	60.4		
55	34	F	28.5	1	10.1	15	67.3		
56	30	F	20.8	0.8	9	10.5	85.7		
57	50	F	17.1	0.9	4.1	6.7	61.1		
58	31	F	23.1	0.8	6.7	12.4	54.03		
59	38	F	34.4	1.1	9.1	18.2	50		
60	30	F	13.4	1	4.9	8.9	55		
61	50	F	13.7	0.9	3.1	6.2	50		
62	57	F	11.02	1.14	3.2	4.9	65.3		
63	30	F	13.6	0.9	4.6	5.3	86.7		
64	26	F	21.6	0.8	14.6	21.1	69.1		
65	33	F	15.6	0.9	5.9	8.4	70.2		
66	55	F	26.3	0.8	10.8	11.7	92		
67	44	F	77.6	0.8	9.6	18.4	52.1		
68	45	F	10.2	0.8	1.8	2.6	69		
69	42	F	96	0.8	41	52	78		
70	55	F	26.3	0.8	8.1	14.1	57		
71	44	F	77.6	0.8	9.6	18.4	52.1		
72	45	F	10.2	0.8	1.8	2.6	69.2		
73	42	F	94	0.8	41.1	52	79		
74	56	M	26.1	1.1	8.1	14	57.8		
75	50	F	39.4	0.8	18.2	20.1	90.5		

76	30	F	16.4	0.8	7.3	8.2	89.02		
77	55	F	12.7	0.8	4.4	6.3	69.8		
78	15	F	92	0.8	40.2	51.6	77.9		
79	49	M	14.5	0.8	5.1	7.2	70.8		
80	63	F	14.4	0.8	4.2	7.5	56		
81	29	M	12.8	0.8	3.1	6.5	47		
82	45	M	44.5	0.9	10	20.1	49.7		
83	40	F	91	0.8	25.7	51.1	50.2		
84	32	F	62.2	0.8	2.8	31.3	**8.9**	5.01	16
85	32	F	28.6	0.8	7.9	13.7	57.6		
86	54	M	14.9	1.08	2.5	5.08	49		
87	34	M	13.5	0.9	2.7	8.86	30.6		
88	34	M	29	0.9	9.01	18.15	49.5		
89	52	F	10.8	1.2	3.3	5	66		
90	47	F	74.1	0.8	32.7	41.5	78		
91	45	F	97	0.8	58.4	83.2	70.1		
92	67	F	35.9	0.8	14.7	22.6	65		
93	40	F	10.2	0.8	4.18	6.1	67		
94	10	F	96	0.8	20.1	48.6	41		
95	45	F	18.7	1.2	4.2	8.5	48		
96	23	F	75.9	0.9	20	35	57		
97	37	F	14.5	1.2	7.2	8.7	82		
98	22	F	98	0.8	37.3	48	77		
99	44	F	57.9	0.8	20.7	25	82		
100	35	F	11.7	0.8	4.1	5.5	74		
101	57	M	11.2	0.9	5.5	6.1	90		
102	55	F	18.8	1.1	8.1	10	81		
103	45	F	31.8	0.8	14.1	15.2	92		

104	37	F	11.2	0.9	4.1	5.5	74		
105	40	F	61.4	0.8	20.7	30.1	68		
106	32	F	81.9	0.8	45	46.1	97		
107	40	F	15.9	1.1	4.3	8.5	50		
108	41	F	15.9	0.9	7.4	8.5	87		
109	40	F	76.6	0.8	30	35.1	85		
110	30	M	12.4	0.9	5.8	6.1	95		
111	60	F	73	0.9	23.7	31.2	75.9		
112	51	M	19.4	0.9	7.1	10.7	63.3		
113	30	F	16.2	1.04	5.3	8.1	65.4		
114	40	M	14.2	1	6.1	7.4	82.4		
115	40	M	25.1	1	11.3	12.6	89.6		
116	50	F	39.4	0.8	9.8	19.5	50.2		
117	60	M	11.2	0.9	3.1	5.1	60		
118	47	F	13.1	0.9	4.3	6.3	68.2		
119	43	F	25.1	1.2	9.04	12.1	74.7		
120	5	F	15.5	1.4	5.8	7.4	78.3		
121	37	F	44.5	0.8	33.6	44.8	75		
122	22	F	64.6	0.8	48.8	66.9	72		
123	22	F	95	0.8	87.17	98.1	88		
124	29	F	98	0.8	86.7	98.4	88		
125	24	F	10.4	1.4	5.2	8.9	58.4		
126	37	F	32.9	0.8	17.13	26.1	65		
127	36	F	37.8	0.8	16.8	18.8	89		
128	35	F	99	0.8	63.3	98.6	64		
129	34	F	98	0.8	9.8	28.02	34		
130	22	F	11.8	0.9	0.69	1.28	53		
131	36	F	14.9	0.8	9.4	10.4	90.3		

132	37	F	10.9	1	5.7	9.9	57.5		
133	27	F	10.4	1.2	4.8	5.9	81.3		
134	37	F	22.4	0.8	4.6	12.5	36.8		
135	34	F	14.4	1.5	1.8	4.3	41.8		
136	32	F	17.4	0.9	2.3	4.7	48.9		
137	28	F	12.6	1.1	1.15	6.8	**17**	**1.7**	25%
138	27	F	35.9	0.8	8.49	22.18	38		
139	37	F	98.6	0.8	23.9	26.12	91.5		
140	17	F	10.6	0.9	2.6	3.03	85.8		
141	37	F	22.6	1.2	14.5	26.8	54.1		
142	32	F	42.9	0.8	14.7	33.9	43.6		
143	27	F	97.6	0.8	68.01	85.5	79.5		
144	65	M	97.7	0.8	24.09	35.1	68.6		
145	31	F	37.1	0.8	12.06	16.02	75.2		
146	36	F	98.8	0.8	90.5	92	98.9		
147	38	F	14.8	0.8	8.15	8.6	94		
148	28	F	91	0.8	53.9	98.7	54.6		
149	58	F	39	0.8	18.1	26.2	69		
150	45	F	15.9	1	8.02	12.6	63.6		
151	44	F	98	0.8	60.5	80.1	76.02		
152	39	F	96	0.9	51.2	60.7	84.3		
153	42	F	23.3	1	9.5	13.2	71		
154	35	F	65.4	0.8	12.8	14.4	88.8		
155	20	M	10.5	0.9	6.03	6.9	87		
156	50	F	20.2	1.1	2.9	4.1	70.7		
157	51	F	13.6	0.8	6.6	11.7	56.4		
158	50	M	31.4	0.8	12.4	15.2	81.5		
159	13	F	98	0.9	18.7	24.8	75.4		

160	50	F	99.2	0.8	40.3	52.2	77.2		
161	29	F	76.1	1	2.2	7.05	31.2		
162	30	F	35	0.8	7.6	14.9	51		
163	27	F	16.5	0.8	1.7	2.7	62.9		
164	43	F	17.9	1.5	2.3	3.4	67.6		
165	31	F	21.5	0.9	4.6	7.9	58.2		
166	34	F	42.7	1.1	13.7	20.5	66.8		
167	40	F	12.8	1.5	3.5	8.9	39.3		
168	21	F	12	1.1	6.2	15.6	39.7		
169	45	F	12.8	1.1	1.02	6.4	**16**	**1.34**	20%
170	22	F	14.7	1.4	2.4	5.7	42.1		
171	55	F	28.1	1.6	3.12	14.2	**22**	6.8	48.00%
172	14	M	94	0.9	44.3	61.9	71.5		
173	20	M	11.1	1	1.5	3.14	47.7		
174	25	F	13.7	1.3	1.1	3.6	30.5		
175	35	F	16.2	1.3	3.2	3.6	88.8		
176	56	M	38.7	0.9	4.6	11.06	41.5		
177	30	F	21.8	0.9	6.19	18.08	34.2		
178	32	F	11.9	1.6	0.89	5.13	**17.3**	1.8	35.00%
179	39	F	30.3	1.4	38.4	60.17	63.8		
180	50	f	13.6	1	1.5	5.2	28.8		
181	39	F	30.3	1.4	38.4	60.17	63.8		
182	35	F	98.7	0.9	43.5	66.1	65.8		
183	55	F	61.5	0.9	15.7	30.2	51.9		
184	45	F	28.7	0.9	9.9	31.08	31.8		
185	32	F	10.1	0.9	1.9	4.2	45.2		
186	22	F	25.7	1	8.09	12.3	65.7		
187	46	F	17.7	0.8	5.7	8.4	67.8		

188	56	F	12	1	2.1	4.2	50		
189	38	F	40.5	0.9	8.4	13.9	60.4		
190	63	F	23.4	0.9	5.3	10.8	49.07		
191	6	F	10.5	1	1.8	2.4	75		
192	60	F	97	0.8	48	59	81.3		
193	32	F	23.4	0.8	8.4	13.3	63.1		
194	45	F	11.5	0.8	2.9	6.7	43.2		
195	18	F	16.1	0.9	6.3	11.2	56.2		
196	50	F	97	0.9	50.1	56.6	88.5		
197	33	F	12.1	0.8	2.4	3.1	77.4		
198	35	F	18.4	1	6.8	12.4	54.8		
199	40	F	18.6	0.8	4.4	6.07	72.4		
200	25	F	72.9	0.9	28.1	48.5	57.9		

PROFORMA

Name: Age: Sex:

Address: Ph. No:

Occupation: Income: Religion:

PAST MEDICAL HISTORY

Hypertension /IHD/Stoke/Trauma/Recent Surgeries

Drug Intake:

Family History: DM/HT/IHD

Personal History: Diet, Veg / Non Veg

Smoking: Average No. of beedies / Cigarettes/Day/ year

Alcohol: Quantity consumed / day x Duration in years

GENERAL EXAMINATION

Built and Nourishment:
Pallor / Cyanosis / Clubbing / Edema / Jaundice / Lymphadenopathy

VITAL PARAMERTERS

PULSE: BP:

SYSTEMIC EXAMINAITON

CVS: RS: P/A: CNS:

INVESTIGATIONS:

TSH

GOVT. STANLEY MEDICAL COLLEGE,\CHENNAI- 600 001

INFORMED CONSENT

EVALUATION OF AN IMMUNOGLOBULIN .G COMPLEXED FORM OF THYROID STIMULATING HORMONE[MACRO TSH] AS INTERFERENCE IN TSH ASSAY

Place of study: Govt. Stanley Medical College, Chennai

I ..... have been informed about the details of the study in my own language.

- I have completely understood the details of the study.
- I am aware of the possible risks and benefits, while taking part in the study.
- I understand that I can withdraw from the study at any points of time and even then, I can receive the medical treatment as usual.
- I understand that I will not get any money for taking part in the study.
- I will not object if the results of this study are getting published in any medical journal, provided my personal identity is not revealed.
- I know what I am supposed to do by taking part in this study and I assure that I would extent my full cooperation for this study.

Volunteer :

Name and address :

Signature :

Date :

Investigator :

Signature and date :

தகவல் படிவம்

மதிப்பிற்குரிய ஐயா / அம்மையீர்,

உங்கள் விருப்பத்தின் பேரில் **"தைராய்டு சுரப்பி குறைபாடு உள்ளவர்களின் இரத்தத்தில் மேக்ரோ டி.எஸ்.எச் குறுக்கீட்டை கண்டறிவதற்கான ஆய்வு"** பற்றிய ஆய்வில் பங்கேற்கும்படி அன்புடன் கேட்டுக்கொள்கிறோம். இந்த ஆய்வில் ஆராய்ச்சி நோக்கத்துக்காக தாங்கள் பரிசோதனைக்கு உட்படுத்தபடுவீர்கள். தகுந்த சிகிச்சை தங்களுக்கு தொடங்கப்படும். தங்களுக்கு இந்த ஆய்வில் பங்கேற்க விருப்பம் இருந்தால் தாங்கள் அருள்கூர்ந்து ஒப்புதல் படிவத்தைப் படித்துக் பார்த்துக் கையொப்பம் இடும்படிக் கேட்டுக் கொள்கிறேன்.

சுய ஒப்புதல் படிவம்

ஆய்வு செய்யப்படும் தலைப்பு

தைராய்டு சுரப்பி குறைபாடு உள்ளவர்களின் இரத்தத்தில் மேக்ரோ டி.எஸ்.எச் குறுக்கீட்டை கண்டறிவதற்கான ஆய்வு

ஆராய்ச்சி நிலையம் : அரசு ஸ்டான்லி மருத்துவமனை, சென்னை,

பங்கு பெறுபவரின் பெயர் :

பங்கு பெறுபவரின் எண் :

பங்குபெறுபவர் ( ) இதனைக் குறிக்கவும் :

மேலே குறிப்பிடப்பட்டுள்ள ஆய்வின் விவரங்கள் எனக்கு விளக்கப்பட்டது. என்னுடைய சந்தேகங்களைக் கேட்கவும், அதற்க்கான தகுந்த விளக்கங்களைப் பெறவும் வாய்ப்பளிக்கபட்டது.நான் இவ்வாய்வில் தன்னிச்சையாகத்தான் பங்கேற்கிறேன். எந்த காரணத்திலோ எந்த கட்டத்திலும் எந்த சட்டச்சிக்கலுக்கும் உட்படாமல் நான் இவ்வாய்வில் இருந்து விலகிக் கொள்ளலாம் என்றும் அறிந்து கொண்டேன். ☐

இந்த ஆய்வு சம்பந்தமாகவோ, இதைச் சார்ந்த மேலும் ஆய்வு மேற்கொள்ளும்போதும் இந்த ஆய்வில் பங்கு பெறும் மருத்துவர் என்னுடைய மருத்துவ அறிக்கையை பார்ப்பதற்கு என் அனுமதி தேவையில்லை என அறிந்து கொள்கிறேன். நான் ஆய்வில் இருந்து விலகிக் கொண்டாலும் இது பொருந்தும் என அறிகிறேன். ☐

இந்த ஆய்வு மூலம் கிடைக்கும் தகவல்களையும் பரிசோதனை முடிவுகளையும் மற்றும் சிகிச்சை தொடர்பான தகவல்களையும் மருத்துவர் மேற்கொள்ளும் ஆய்வில் பயன்படுத்திக் கொள்ளவும் அதைப் பிரசுரிக்கவும் என் முழுமனதுடன் சம்மதிக்கிறேன். ☐

இந்த ஆய்வில் பங்கு கொள்ள ஒப்புக் கொள்கிறேன். எனக்கு கொடுக்கப்பட்ட அறிவுரைப்படி நடந்து கொள்வதுடன் இந்த ஆய்வை மேற்கொள்ளும் மருத்துவ அணிக்கு உண்மையுடன் இருப்பேன் என்று உறுதி அளிக்கின்றேன். என் உடல் நலம் பாதிக்கப்பட்டலோ அல்லது எதிப்பாராத வழக்கத்திற்கு மாறான நோய்க்குறி தென்பட்டாலோ உடனே அதை மருத்துவ அணிக்குத் தெரிவிப்பேன் என உறுதி அளிக்கிறேன். ☐

பங்கு பெறுபவரின் கையொப்பம்_____இடம்_____ தேதி_____

கட்டைவிரல் ரேகை

பங்குபெறுபவரின் பெயர் மற்றும் விலாசம்_____

ஆய்வாளரின் கையொப்பம்_____இடம்_____ தேதி_____

ஆய்வாளரின் பெயர் **மரு. ச. அர்ச்சனா தேவி**

INSTITUTIONAL ETHICAL COMMITTEE, STANLEY MEDICAL COLLEGE, CHENNAI-1

Title of the Work : Evaluation of Immunoglobulin G complexed form of Thyroid stimulating hormone (Macro TSH) as interference in TSH assay.

Principal Investigator : Dr. C Archana Devi

Designation : P G MD (Bio-Chemistry)

Department : Department of Bio-Chemistry
Government Stanley Medical College,
Chennai-01

The request for an approval from the Institutional Ethical Committee (IEC) was considered on the IEC meeting held on 12.01.2017 at the Council Hall, Stanley Medical College, Chennai-1 at 2PM

The members of the Committee, the secretary and the Chairman are pleased to approve the proposed work mentioned above, submitted by the principal investigator.

The Principal investigator and their team are directed to adhere to the guidelines given below:

1. You should inform the IEC in case of changes in study procedure, site investigator investigation or guide or any other changes.
2. You should not deviate from the area of the work for which you applied for ethical clearance.
3. You should inform the IEC immediately, in case of any adverse events or serious adverse reaction.
4. You should abide to the rules and regulation of the institution(s).
5. You should complete the work within the specified period and if any extension of time is required, you should apply for permission again and do the work.
6. You should submit the summary of the work to the ethical committee on completion of the work.

MEMBER SECRETARY, 8/3/17
IEC, SMC, CHENNAI

MEMBER SECRETARY
ETHICAL COMMITTEE,
STANLEY MEDICAL COLLEGE
CHENNAI-600 001.

CERTIFICATE-II

This is to certify that this dissertation work titled **"EVALUATION OF IMMUNOGLOBULIN G COMPLEXED FORM OF THYROID STIMULATING HORMONE [MACRO TSH] AS INTERFERENCE IN TSH ASSAY"** of the candidate **Dr. C.ARCHANA DEVI** with registration number **201523051** for the award of **M.D. BIOCHEMISTRY** in the branch of **XIII**. I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows **1%** percentage of plagiarism in the dissertation.

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